University of Alberta

The ecology of *Armillaria sinapina* in mixedwood forests of north-western Alberta, Canada

by

Lisa M. Cuthbertson ©

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Abstract

The purpose of this study was to examine the ecology of *Armillaria sinapina* in boreal mixedwood forests in north-western Alberta. Nine 40 × 40 m plots were established in stands that ranged from 7.2% to 92% broadleaf trees, snags, and stumps. Using logistic regression, I determined which variables best predicted the presence of *A. sinapina* in trees, snags, stumps, downed woody material, forest floor cores, and trap-logs. Second, I described the spatial point pattern of the fungus infecting trees, snags, and stumps. The main results were that *A. sinapina* preferred broadleaf substrates over coniferous substrates. Dead coniferous trees were more likely to be infected by *A. sinapina*. This result suggests that *A. sinapina* may be playing a role in the death of coniferous trees. The spatial point pattern of infected trees, snags, and stumps reflected the underlying spatial point pattern of the host trees.

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Chapter one

General introduction

This thesis is a study of the species *Armillaria sinapina* Bérubé and Dessureault. This first chapter begins by giving an introduction to *Armillaria* by describing the world-wide distribution and the different hosts that are infected by the members of the genus, the identification techniques that have been used to differentiate between the different species within the genus, and the morphology and infection characteristics of the genus. Next, the ecological roles of *Armillaria*, dispersal characteristics, and what is known to date about the spatial ecology of the genus are reviewed. In addition, the application of *Armillaria* ecology to forest management issues is discussed, and what is known to date about the ecology of *A. sinapina*. The objectives and hypotheses of the thesis are listed. Lastly, the general methodologies used in the study are described.

Distribution and hosts

Species in the genus *Armillaria* (Fr.:Fr.) Staude are basidiomycete fungi belonging to the order Agaricales and in the family Tricholomataceae. *Armillaria* commonly cause root disease in many economically important plant species throughout the world (Wargo and Shaw 1985, Ota *et al.* 1998). Economic crops susceptible to *Armillaria* are numerous and the following examples are but a short list of such plant species: tea (Butler 1928, Leach 1939, Onsando *et al.* 1997), pear orchards (Rizzo and Whiting 1998), cocoa (Dade 1927, from Swift 1968), and conifer plantations in Eastern and Central Africa (Swift 1972), New Zealand (MacKenzie and Shaw 1977, van der Pas 1981), and Japan (Ota *et al.* 1998). In addition, to causing disease in crops, *Armillaria* occurs world-wide in boreal, temperate, and tropical forests causing disease of both coniferous and broadleaf trees (Wargo and Shaw 1985, Kile *et al.* 1991). In Canada, *Armillaria* is present in most forest regions (Morrison *et al.* 1985, Bérubé and Dessureault 1988, 1989, Dumas 1988, Mallett 1990, 1992, Bérubé 2000). In Alberta, there are two known species of *Armillaria*: *Armillaria ostoyae* (Romagn.) Herink, which is known to cause disease in juvenile coniferous trees in North America (Wargo and Shaw 1985, Mallett 1990, Morrison *et al.* 2000) but is also found on a wide variety of coniferous and broadleaf hosts (Mallett 1990) and *Armillaria sinapina*, which is frequently associated with broadleaf hosts and has rarely been isolated from coniferous hosts (Mallett 1990).

Morphology and infection

The basidiocarps (mushrooms) of *Armillaria sinapina* have a yellow to golden brown cap, the gills are cream coloured and there is an annulus on the upper portion of the stipe (stem) (Bérubé and Dessureault 1988) (Figure 1.1a). In culture, *A. sinapina* starts out as a white and fluffy mycelia and over time forms a reddish-brown to dark brown crust over the mycelia. *Armillaria sinapina* produces abundant light to dark brown monopodially branching rhizomorphs (Bérubé and Dessureault 1988), which are tubular aggregates of mycelia with a outer coating of melanized hyphae (Figure 1.1b). These rhizomorphs have sharp tips and can grow rapidly through the forest floor until they encounter a suitable substrate such as a root, root collar or piece of downed woody material.

Once the rhizomorphs have infected the roots and/or root collars of a suitable host, subcortical fans of white mycelium develop in the cambium of roots and root collars (Figure 1.1c). Pseudosclerotial plates, melanized mycelia which are dark coloured, are often present giving the characteristic black zone lines which are easily seen by cutting longitudinally into the roots and root collars of infected trees, snags, and stumps as well as in downed woody material (Figure 1.1c and d). In well-decayed substrates, *Armillaria* species causes a yellow-stringy rot of the wood, a form of white rot. White rot fungi digest cellulose and lignin in the cell walls of wood making the wood soft, spongy and whiter than normal wood (Figure 1.1c). As opposed to brown rot fungi that digest the cellulose and leave the lignin behind rendering the wood dry, brittle and darker than normal wood.

In this study, *Armillaria sinapina* was recorded at the root collar and on roots of coniferous and broadleaf hosts. Infecting rhizomorphs of *A. sinapina* were characterised by penetration of roots and root collars and spread into the cambial region in the form of subcortical fans of white mycelium (mycelial fans) (Figure 1.2).

Dispersal

There are three different means by which *Armillaria* species are able to disperse: (1) by haploid basidiospores produced by the basidiocarps, (2) by rhizomorphs, or (3) root-to-root contact through the host plants. Spore production by *Armillaria* basidiocarps is immensely prolific; up to1,000 viable basidiospores per dm^2 per minute under the basidiocarp (Rishbeth 1970). Generally however, rhizomorphs, rather than basidiospores are thought to play the primary role in dispersal at the local scale. This is especially true for systems with a high density of *Armillaria* and presence of rhizomorphs (Swift 1968). Hartig in 1873 was the first to suggest that rhizomorphs of *Armillaria* were responsible for the spread and infection of the disease in a conifer plantation (Redfern and Filip 1991). Experimental studies with two-year-old pine saplings planted varying distances from a centrally located infected tree demonstrated that roots of saplings that came into contact with the central infected pine saplings became infected (Swift 1968). Based on experimental studies Rishbeth (1964) showed that *Armillaria mellea* seldom infected stumps by basidiospores but infected stumps by rhizomorphs or by root-to-root contact. Other studies have shown that *Armillaria sinapina* form rhizomorphs more abundantly than does *Armillaria ostoyae* (Rishbeth 1985, Blodgett and Worrall 1992, Banik *et al.* 1995). However, some researchers have suggested that the spread of *Armillaria* could also be by root-to-root contact and that rhizomorphs were playing a secondary role in infection (Swift 1968). Previous study has suggested that root-to-root infection may be typical in some coniferous forests with highly pathogenic species of *Armillaria*, such as *A. ostoyae*, that produce few rhizomorphs (Morrison *et al.* 1985).

Identification techniques and taxonomy

Understanding the biology, pathogenicity, and ecology of *Armillaria* has been confused by the previously widespread acceptance of *Armillaria mellea* (Vahl:Fr.) Kummer as a highly polymorphic species found world-wide (Mallett 1990, Kile *et al.* 1991). Up until the late 1970's, this led to a consensus that the fungus was extremely variable in its pathogenicity and its host range (Watling *et al.* 1991). After the recognition of the biological species concept (Anderson and Ullrich 1979), researchers began to question whether this complex was actually a group of closely related species. Following this, the *A. mellea* complex has been broken up into many different species.

There are several different identification methods that have been used to distinguish between the species of *Armillaria*: (1) haploid-haploid pairings, where two colonies are paired and either remain white and fluffy, meaning they were two different species, or fuse and become a dikaryotic crustose colony meaning they were two of the same species (Hintikka 1973) (2) haploid-diploid pairings, where two colonies are paired and the haploid isolate becomes crustose (diploid) if they were from the same species or remains fluffy if they are not (Korhonen 1978, Morrison *et al.* 1985, Blodgett and Worrall 1992, Harrington and Rizzo 1993) (3) diploid-diploid pairings, where a black line (melanized hyphae) forms between pairs that are different species (Mallett and Hiratsuka 1986) and (4) molecular methods such as isoenzyme patterns (Morrison *et al.* 1985), restriction fragment patterns of mitochondrial DNA (Jahnke *et al.* 1987), and restriction fragment patterns of nuclear ribosomal DNA (Anderson *et al.* 1989, Harrington and Wingfield 1995). Harrington and Wingfield (1995) developed a PCR-based technique, which has made for quick and reliable identifications of *Armillaria* species from culture, sporocarps, rhizomorphs and colonized wood. Their method relies on the amplification of the intergenic spacer region (IGS) of nuclear ribosomal DNA.

In Europe, Korhonen (1978) used the haploid pairing technique to separate *Armillaria mellea* collections into five intersterile groups, termed 'biological species'. Roll-Hansen (1985) further divided *Armillaria* species in Europe into six intersterile groups. Currently, in Europe, there are seven species of *Armillaria* recognised (Guillaumin *et al.* 1991). All of these European biological species have been linked with their corresponding morphological species therefore the biological species are now considered taxonomic species (Guillaumin *et al.* 1991). In Australasia, five biological species of *Armillaria* have been linked with their morphological species based on morphology of the sporocarps, vegetative morphology in culture, and mating tests (Guillaumin *et al.* 1991). In Japan, up to eight biological species have been reported (Terashima *et al.* 1998). In North America, Anderson and Ullrich (1979) used the haploid pairing technique to distinguish biological species of *Armillaria.* There are up to eleven intersterile groups of *Armillaria* which are often referred to as North American Biological Species (NABS) with an assigned roman numeral I - XI (Banik *et al.* 1996, Mallett 1990, Morrison *et al.* 1985). The NABS assignment for the two species of *Armillaria* in Alberta are NABS I = *A. ostoyae* and NABS V = *A. sinapina* (Bérubé and Dessureault 1988). Most of these biological species of *Armillaria* have been linked with morphological taxa and are now referred to by their Latin names (Bérubé *et al.* 1996). To date, there have been up to 30 biological species of *Armillaria* reported throughout the world (Terashima *et al.* 1998).

Using restriction enzymes such as *Alu*I digests of the amplified product, unique restriction fragment length ploymorphisms (RFLPs) have been identified for all North American *Armillaria* species except *A. gallica* from *A. calvescens* (Banik *et al.* 1996, Shulze *et al.* 1997). There are distinct banding patterns recognised for two of the most common *Armillaria* species in the western mixedwood boreal forest where this study took place: *Armillaria ostoyae* and *A. sinapina* based on the restriction enzyme *Alu*I (see Harrington and Wingfield 1995, Colin Myrholm, personal communication, 2000). It was using these methods that the taxon considered in this thesis was identified: *Armillaria sinapina*.

Pathogenicity and disease

Some research has been conducted to address the pathogenicity of *Armillaria* species and how they cause disease in host plants. Pathogenicity refers to "the quality or characteristic of being able to cause disease" (British Federation of Plant Pathologists 1973). A plant host, which is infected, is usually said to be diseased only when symptoms become evident. Disease is defined as a deviation from normal functioning of physiological processes in the host (British Federation of Plant Pathologists 1973). A pathogen is defined as an entity that can incite disease.

Disease is manifested through the expression of symptoms in the host. There are several symptoms of the disease caused by *Armillaria*. Coniferous hosts may show loss of needles (pine needles turn dull green, then yellow, then red whereas spruce needles turn dull green but rarely yellow or redden before the needles drop off), excess resin production at the root collar (resinosis), growth loss, and excess cone production (Mallett 1992). Symptom expression of the disease in broadleaf hosts is typically less obvious and may include yellowing of the leaves and crown death.

Armillaria spp may act synergistically with other factors that cause tree disease and death. *Armillaria* root disease has been found to be associated with insects such as bark beetles and jack pine budworm (Mallett and Volney 1990, Kile *et al.* 1991).

Spatial ecology

One of the most interesting features of *Armillaria* species ecology is the variation in its distribution in a forest. Hartig in 1873 was the first to state that the pattern of spread by *Armillaria* in plantations was radial and that *Armillaria* infection was originating from a few localised sources of infection (see Hartig 1873 in Swift 1968). Researchers have found distributions that range from randomly scattered to large (several hectare) 'disease centres' where the species is highly clumped on the landscape (Wargo and Shaw 1985, Kile *et al.* 1991). 'Disease centres', mainly characterised as openings in the forest with dead trees in the centre and with dying trees on the perimeter have been recorded in coniferous stands in North America (Kile *et al.* 1991, Mallett 1992). The size and scale of variation of disease centres is immense ranging from a group of a few dead trees to tens of hectares (Wargo and Shaw 1985, Kile *et al.* 1991).

Forest management implications

Stand composition may affect the patterns of disease caused by *Armillaria* spp. However, little work has been done in this area (Gerlach *et al.* 1997). Alternatively, forest root diseases, such as *Armillaria* spp., may play an important role in forest succession, stand composition and structure in mixed-conifer forests. For example, on wetter sites in the interior forests of British Columbia, *Armillaria ostoyae* is able to kill pioneering tree species such as Douglas-fir and lodgepole pine causing gaps in these forests. These gaps are then revegetated with shade-tolerant tree species such as western hemlock and western red cedar which are still susceptible to *A. ostoyae* infection but mortality is low (Kile *et al.* 1991)

Human-caused disturbances such as clearcutting, precommercial thinning and partial cutting practices may influence the distribution of *Armillaria* root disease. Harvesting may lead to inoculum buildup on many species of conifer stumps in western North American forests (Kile *et al.* 1991). This inoculum is then available and ready to infect young regenerating trees. For all three cutting practices, it is thought that planted and naturally regenerating trees are in danger of increased mortality (Morrison and Mallett 1996). For example, in a 25-year period, a site in Nelson B.C. was subjected to three partial cutting entries. By the third entry, over 90% of the stumps had viable *Armillaria ostoyae* inoculum, which resulted in severe understocking of regenerating trees because most were being killed before they reached merchantable size (Morrison and Mallett 1996). Generally, it is thought that if the *Armillaria* species are aggressive pathogens pre-disturbance then cutting may exacerbate disease expression in the planted or naturally regenerating tree species (Kile *et al.* 1991). Alternatively, if the *Armillaria* disease effects are non-lethal pre-disturbance then the disease expression seems to remain non-lethal from crop to crop (Kile *et al.* 1991). However, little experimental work has been done on the effect of management practices on *Armillaria* disease levels (Kile *et al.* 1991). Forest management strategies to reduce inoculum levels of Armillaria root disease after cutting include physically removing stumps, planting less susceptible tree species, and planting tree species further away from colonized stumps (Morrison and Mallett 1996).

Natural disturbances such as fire may influence the distribution of *Armillaria* root disease. Fire may physically control *Armillaria* activity through destruction of inoculum or indirectly by favouring the growth of other fungi, which may act as a biocontrol of *Armillaria* (Kile *et al.* 1991). For example, isolates of the soil-inhabiting fungus *Trichoderma* spp from burnt soils have been shown to be more antagonistic to *Armillaria* colonies and rhizomorph growth in culture than from unburnt soils (Reaves *et al.* 1990). This suggests that fire may play a role in enhancing biological control of *Armillaria* sp.

The ecology of Armillaria sinapina

Some research has been conducted that gives us clues as to the ecological role of *Armillaria sinapina* in the mixedwood forests of north-western Alberta. To date, it is thought that *A. sinapina* is a weak pathogen of both broadleaf and coniferous trees, that it produces many rhizomorphs in the soils of broadleaf forest stands and that it is more likely to be found associated with broadleaf hosts than coniferous hosts (Morrison *et al.*

1985, Mallett 1990, Banik *et al.* 1995). In contrast, greenhouse inoculation experiments show that *A. sinapina* is an aggressive pathogen causing disease and mortality in coniferous trees (Mallett and Hiratsuka 1988, Mallett pers. comm.). However, it has also been shown in greenhouse inoculation experiments that *A. sinapina* does not kill conifer trees (Mugala *et al.* 1989). In south-eastern Alaska forests, where *A. sinapina* is widely distributed, little killing is evident in regenerating stands of sitka spruce and Alaska-cedar (Shaw and Loopstra 1988). Further, the authors suggest that the presence of *A. sinapina* and another weak pathogen NABS IX in stump and root wood might act as a biocontrol of another root rot pathogen, *Heterobasidion annosum*, by deterring its spread from the stumps to adjacent trees. Knowledge of its presence in downed woody material and in healthy asymptomatic trees is virtually unknown. To date, there have not been any empirical field studies that look directly at what environmental factors are important for the presence of *A. sinapina*.

Objectives

The overall goal of this thesis research was to investigate the ecology of *Armillaria sinapina* in the mixed-wood boreal forest on a small scale in trees, snags, stumps, downed woody material, and in the soil. The specific objectives were: (1) To identify the predictors of the occurrence of *A. sinapina* in trees, snags, and stumps, in downed woody material, and in the forest floor. In Chapter Two, the occurrence of *A. sinapina* will be related to measured environmental variables for each substrate type sampled. (2) To identify the spatial pattern of *A. sinapina* in trees, snags, and stumps. In Chapter Three a spatial analysis was used to determine the small scale pattern of infection of trees, snags, and stumps. Chapter Four will synthesise the results and conclusions from the two previous chapters, put them in context with the current literature and propose future work and improvements to the methods employed in this study.

General methods

Study area

This current study was part of a large-scale forestry experiment, which is trying to determine which forest harvest and regeneration practices best maintain biotic communities, as compared to those resulting from natural disturbances, such as fire. Sampling took place at the EMEND (Ecological Management Emulating Natural Disturbance) field research site, located at township 89-90, range 03, west of the 6th meridian, 90km Northwest of Peace River, Alberta. This research site is located on a joint forest management area managed by Canadian Forest Products (CanFor) Ltd. and Daishowa-Marubeni International (DMI) Ltd. The area is characterised by mixed coniferous and broadleaf forests. All sampling in this current study was conducted only in the uncut control blocks of the EMEND experimental area.

This forest region has a mean annual precipitation of 570mm and a mean May to September temperature of 11.5°C (Strong and Leggat 1992). The area is characterised by mixedwood forests occurring on medium-textured, moderately well-drained Gray Luvisolic soils (Strong and Leggat 1992). The dominant forest type is a naturally regenerated mixture of aspen (*Populus tremuloides* Michx.), balsam poplar (*Populus balsamifera* L.), white spruce (*Picea glauca* (Moench) Voss), and black spruce (*Picea mariana* (Mill.) BSP.). Tree composition of stands varies widely in the boreal mixedwood forests. Mixed stands of aspen and white spruce are typical of much of the boreal mixed-wood region but pure stands of each species exist. Tree composition can vary from almost-pure white spruce stands to mixed-wood to aspen-dominated stands which have a few young white spruce in the understory.

Data collection

Site selection

In late April 1999, nine 10 ha stands at the EMEND research site were selected. The criteria for selecting stands were: minimal to 0° slope, minimal understory shrub vegetation, and at least 50 metres away from large human made openings (roads, cut areas). Nine separate stands were selected ranging in broadleaf canopy tree composition from very low to very high amounts. All stands were "mature" ranging in age from ~ 90 to 120 years (see Appendix I for more details on stands). Using maps of the stands, an area was delineated within each stand, that met the criteria above, and a grid of points spaced 5 m apart was overlayed on the map and used to randomly select each plots' south-west corner starting point. At all south-west corner starting points a rebar stake was driven into the ground. Plots were laid out from this stake 40 m to the north north and 40 m to the east.

All trees, snags and stumps were mapped (to within 1 m) in the 40 × 40 m plots (total of 1911 substrates sampled) by measuring the distance and direction to each substrate from transects running east-west and placed every 2 m throughout the plot. Five 40 m transects (west to east) were placed through the plots every 10 m to sample the downed woody material (total of 455 logs sampled at the intercept and 327 sampled at the base). Sixteen forest floor cores were taken to sample for presence and abundance of rhizomorphs, 16 more forest floor cores were taken for determining soil moisture, and 16 resin bags were placed on a 10 m grid within the plot to assess nutrient availability and 25 trap logs were staked on a separate 10 m grid and 8 trap logs were staked on a central 1 m grid to sample for the presence of rhizomorphs (Figure 1.3)

Infected trees, snags, and stumps

Within each plot, all living coniferous and broadleaf trees greater than 5 cm in diameter at breast height, all snags over 1.3 m in height and stumps under 1.3 m in height were mapped to obtain their relative spatial positions (X, Y) and examined for the presence of *Armillaria* sp. Environmental variables were collected for all trees, snags and stumps, such as tree species and diameter at breast height (Table 1.1).

Coniferous trees, snags and stumps were inspected for the presence of *Armillaria* sp. at the root collar and two roots per tree. Two primary roots were exposed and inspected to 0.5 m from the root collar. In each plot, 5% of the conifer trees were further inspected, by exposing all the main roots from the root collar to 0.5 m, confirming that results from the two-root inspection were reliable in identifying the presence of *A. sinapina* in the tree.

For the majority of broadleaf trees, the roots could not be exposed up to 0.5 m because they grew down into the mineral soil. For these, the root collar region and as much of the roots as possible were inspected for the presence of *Armillaria* sp. Rhizomorphs were followed along the outer bark of the root collar and roots and into the cambium. Where present, formation of a mycelial fan was noted. 'Infected' was where the rhizomorphs penetrated the outer bark and grew into a mycelial fan in the cambium area.

Colonized downed woody material (DWM)

Downed woody material (DWM), greater than 7.5 cm in diameter at the intercept point, that was intercepted by the transect was inspected for the presence of *Armillaria* on five 40 m transects per plot. The DWM was labelled as colonized if the fungus was present under the bark, or inside the wood if the bark was missing, in the form of rhizomorphs, a melanized black zone-line, or a mycelial fan (Figure 1.1d).

Environmental variables, thought to be potentially important for fungal occurrence in DWM were recorded at each log intercept, such as DWM species, and diameter (Table 1.1). In addition, the bearing from the intercept to the base of the log (direction of fall), the length from the intercept to the base, and presence of *Armillaria* at the base, were recorded for pieces of DWM which included the base of the trunk (an intact base).

Presence of rhizomorphs in the forest floor and colonizing trap logs

Cores of the forest floor included the following, the leaf litter layer, the fermenting layer and the humus layer (LFH). The mineral soil was not sampled for the presence of *Armillaria* sp. because it was too hard so estimates of rhizomorph abundance are conservative. Within each of the nine plots, sixteen 10.3 cm diameter cores of the forest floor were taken on a 10 m grid and the LFH layer was measured in centimetres for each core. These forest floor cores were inspected for the presence of rhizomorphs. The length of rhizomorph sections found within each core was also measured and a total rhizomorph length per volume of forest floor was calculated using the core diameter and the depth of the LFH layer. A 5 cm diameter core of the forest floor was taken within 5 cm of the original core and used to measure forest floor moisture. Forest floor (LFH) moisture was determined by weighing the field-fresh sample, followed by drying the LFH samples at 70°C for 48 hours and then reweighing (Karla and Maynard 1991). Moisture was expressed as a percentage by subtracting fresh LFH weight by ovendried LFH weight and dividing by ovendried LFH weight and multiplying by 100 (see Table 1.1, Karla and Maynard 1991).

In mid-May 1999, in each of the plots, sixteen ion-anionic exchange resin bags were placed between the bottom of the surface organic horizon (LFH) and the top mineral horizon on the 10 m grid (as recommended by Dr. Munson pers. comm.) within 5 cm of the forest floor core and the forest floor moisture core. The resin bags were collected in late August. Each bag consisted of a nylon stocking with 35ml of IONAC NM-60 (Baker Scientific) mixed bed exchange resin in preparation for placement in the field. These resin bags were agitated for 1 hour in 2N NaOH, rinsed in deionized water and then agitated in 2N NaCL for one hour. All bags were rinsed with deionized water until the pH of the water was neutral. Bags were stored at 4°C until they were used in the field. Following collection of the bags in August 1999, the bags were stored at 4°C until they were processed in July 2000. Resin was removed from the nylon sacks and 100ml of the extraction solution, 2N NaCL, was added and agitated on a bed shaker for 90 minutes and then filtered (Whatman No. 42). Joe Crumbaugh, a technician at the Northern Forestry Centre, Canadian Forest Service, analysed the resin samples for the following inorganic nutrients, Ca, K, Mg, Mn, S, P, NH_4^+ and NO_3^-. A Technicon AutoAnalyzer II was used to analyse total amount of inorganic nitrogen (NH_4-N and NO_3-N) g/L, for each sample (see Table 1.1, Karla and Maynard 1991). An inductively coupled plasma-atomic emission spectrometer (ICP-AES) was used to analyse total amount of Ca, K, Mg, Mn, S, and P (see Table 1.1, Karla and Maynard 1991).

Trap logs are a method for baiting *Armillaria* from the soil that involves driving small aspen stakes through the forest floor and into the first mineral soil horizon (Mallett and Hiratsuka 1985). Healthy living trembling aspen saplings were selected and cut into trap-logs in May 1999. The logs ranged between 3 to 8 cm in diameter and 20 to 30 cm in length. The trap-log ends were cut with a chainsaw approximately at 45° angles. In early May 1999, in each of the nine plots, thirty-three trap logs were installed on a 10 m grid and a 1 m grid in the plot centre. The trap logs were removed in late August and were inspected for the presence of *A. sinapina*. The trap-log was recorded as being colonized if rhizomorphs were growing along the bark and/or a mycelial fan was growing under the bark.

In mid-August 1999, 0.5 × 0.5 m vegetation quadrats were centred over each resin bag and each trap-log. The percent cover of each plant species, leaf litter, and small woody debris (less than 1 cm in diameter) was estimated using six cover classes: (1) less than 1% cover; (2) 1-5%; (3) 5-25%; (4) 25-50%; (5) 50-75%; (6) 75-100%.

Fungal culturing and identification

For three of the nine plots (Plots 2, 6 and 7) all signs of *Armillaria* were recorded and samples were collected for positive identification of the genus in culture. All *Armillaria* samples were cultured on BDP media, a special media which enhances the growth of *Armillaria* species and suppresses the growth of other fungi and bacteria (Worrall 1991). *Armillaria* samples were identified to genus in culture by the characteristic presence of rhizomorphs, fluffy white mycelia and reddish dark crustose cultures. If the laboratory culture did not grow, the tree was recorded as absent for the presence of *Armillaria*. For three intensively sampled plots, field identification and collections of *Armillaria* (from trees, snags, stumps, downed woody material, trap-logs and forest floor cores) were confirmed to be *Armillaria* species in culture. For the rest of the plots, all field signs of *Armillaria* were recorded but only 5% of *Armillaria* infecting each substrate were collected for positive identification.

To identify *Armillaria* samples to species identifications were done using a PCR-based technique by Harrington and Wingfield (1995), modified by Colin Myrholm at the Northern Forestry Centre, Canadian Forest Service, Natural Resources Canada. DNA was extracted from each *Armillaria* sample to obtain template DNA. The intergenic spacer region (IGS) of the nuclear ribosomal DNA was amplified by polymerase chain reaction (PCR) in a thermal cycler. The *AluI* restriction enzyme was incubated at 37° C with the amplified DNA for 16 hours. This restriction enzyme recognizes and cuts 4bp sequences common in the fungal ITS region (Egger 1995). The digested DNA was loaded on to 1.5% agarose gels and separated by electrophoresis for 1 hour at 75 volts. Known isolates of both *Armillaria sinapina* and *Armillaria ostoyae* were loaded on to the gel for comparison with unknowns and a 50bp DNA ladder used to determine fragment size. Gels were stained with ethidium bromide for 20 minutes, the staining procedure was stopped in distilled water and then gels were viewed under UV light and photographed. Three *Armillaria* samples from each substrate type per plot (total of 81 samples): trees, downed woody material, and forest floor were identified as *Armillaria sinapina*. There was one banding pattern that was typical and found in more than half of the samples analysed (Figure 1.4). This banding pattern is labelled C, which has characteristic bands at 399, 240, 200, 183, and 135 basepairs. However, there were several other less common *A. sinapina* banding patterns also found.

Table 1.1: A list of the environmental variables recorded for trees, snags, and stumps, downed woody material, resin bags, and forest floor cores and trap logs at which *Armillaria sinapina* was recorded. In addition the relative spatial position X, Y of each substrate type was recorded.

Substrate	Name	Variable description
Trees, snags and stumps	Diameter	Basal diameter (m) measured at the root collar.
	Height	Tree height (m)
	Species	Two categories: (1) **broadleaf**: *Populus tremuloides, Populus balsamifera, Betula papyrifera*, and *Salix* spp. and (2) **coniferous**: *Picea glauca, Picea mariana, Pinus contorta, Abies balsamea*, and *Larix laricina*.
	Health	Two categories: (1) **healthy** (2) **dead** (snag or stump) (3) **declining**
Downed woody material	Species	Two categories: (1) **broadleaf**: *Populus tremuloides, Populus balsamifera, Betula papyrifera*, and broadleaf logs; (2) **coniferous**: *Picea glauca, Picea mariana*,and *Picea* sp.
	on/off	Two categories: (1) **on the ground** and (2) **off the ground**
	Diameter	Diameter at the intercept (m)
	Direction of fall	For the base DWM dataset the direction of fall was measured by a compass. Four categories: (1) **0° - 90°** (2) **91° - 180°** (3) **181° - 270°** and (4) **271° - 359°**
Forest Floor	Inorganic nutrients	Availability (using resin bags) of inorganic nutrients (mg/L):Ca, K, Mg, Mn, S, P, NH_4^+, NO_3^-
	Vegetation type	Four Twinspan categories: (1) **Coniferous dry**; (2) **Coniferous moist**; (3) **Broadleaf dry**; (4) **Broadleaf moist**
	Leaf litter	Six abundance classes for leaf litter: (1) **less than 1% cover**; (2) **1-5%**; (3) **5-25%**; (4) **25-50%**; (5) **50-75%**; (6) **75-100%** were recorded in 0.25m^2 quadrats around resin bags
	Small woody debris	Six abundance classes for small woody debris (less than 1 cm in diameter): (1) **less than 1% cover**; (2) **1-5%**; (3) **5-25%**; (4) **25-50%**; (5) **50-75%**; (6) **75-100%** were recorded in 0.25m^2 quadrats around resin bags
	Depth of LFH	A 10.3 cm diameter core was taken 5cm adjacent to the resin bag site and depth of the forest floor (LFH) was measured (cm) and total length and number of rhizomorph pieces were recorded.
	Moisture of forest floor	A 5 cm diameter core (5 cm adjacent to the large forest floor core and the resin bag) of the forest floor was taken to measure moisture: **Wet weight (g) – dry weight (g) /dry weight (g) × 100 = moisture (g)**
Trap-logs	Vegetation type	Four Twinspan categories: (1) **Coniferous dry**; (2) **Coniferous moist**; (3) **Broadleaf dry**; (4) **Broadleaf moist**
	Leaf litter	Six abundance classes for leaf litter: (1) **less than 1% cover**; (2) **1-5%**; (3) **5-25%**; (4) **25-50%**; (5) **50-75%**; (6) **75-100%** were recorded in 0.25m^2 quadrats around trap-logs
	Small woody debris	Six abundance classes for small woody debris (less than 1 cm in diameter): (1) **less than 1% cover**; (2) **1-5%**; (3) **5-25%**; (4) **25-50%**; (5) **50-75%**; (6) **75-100%** were recorded in 0.25m^2 quadrats around trap-log

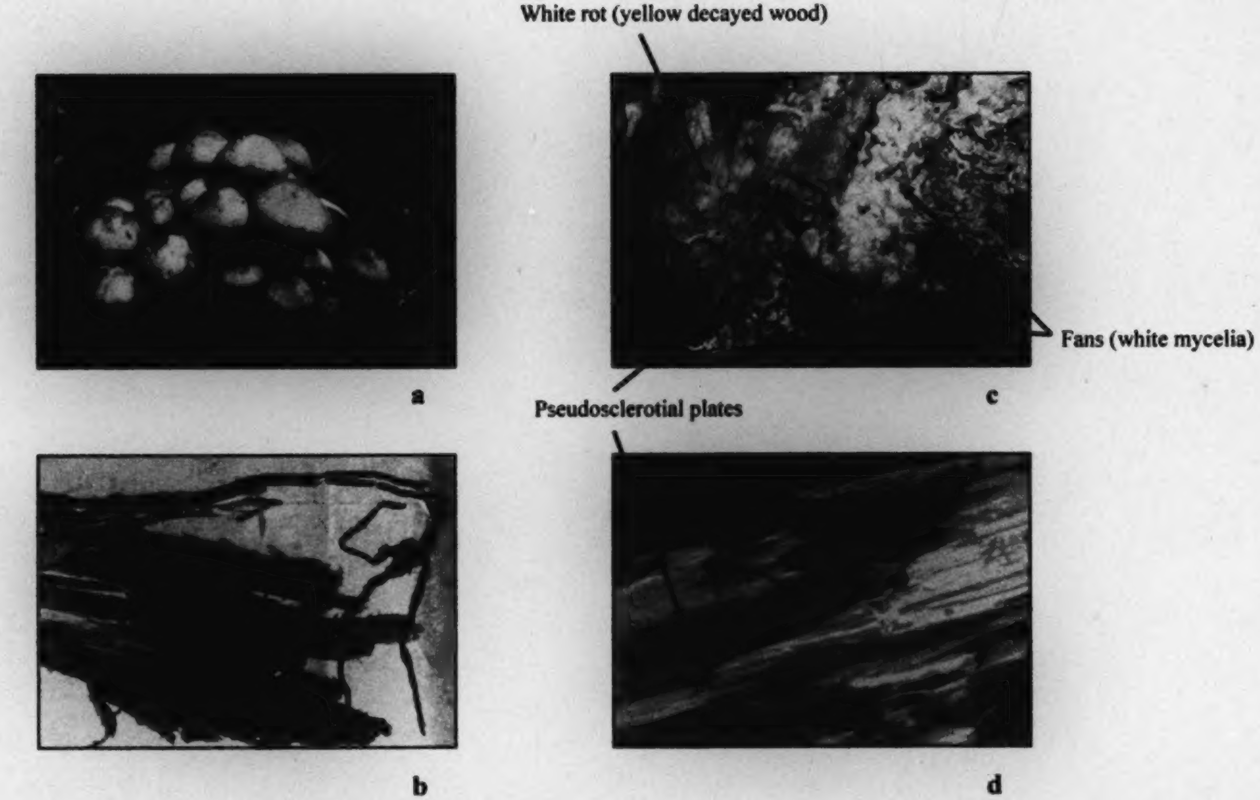
White rot (yellow decayed wood)
Fans (white mycelia)
Pseudosclerotial plates
a
b
c
d

Figure 1.1. Photographs of (**a**) basidiocarps of *Armillaria sinapina*, (**b**) *A. sinapina* rhizomorphs, (**c**) white mycelial fans of *A. sinapina* in a stump, with black pseudosclerotial plates and white rot, which is yellow, stringy, wet wood, and (**d**) black pseudosclerotial plates of *A. sinapina* in downed woody material.

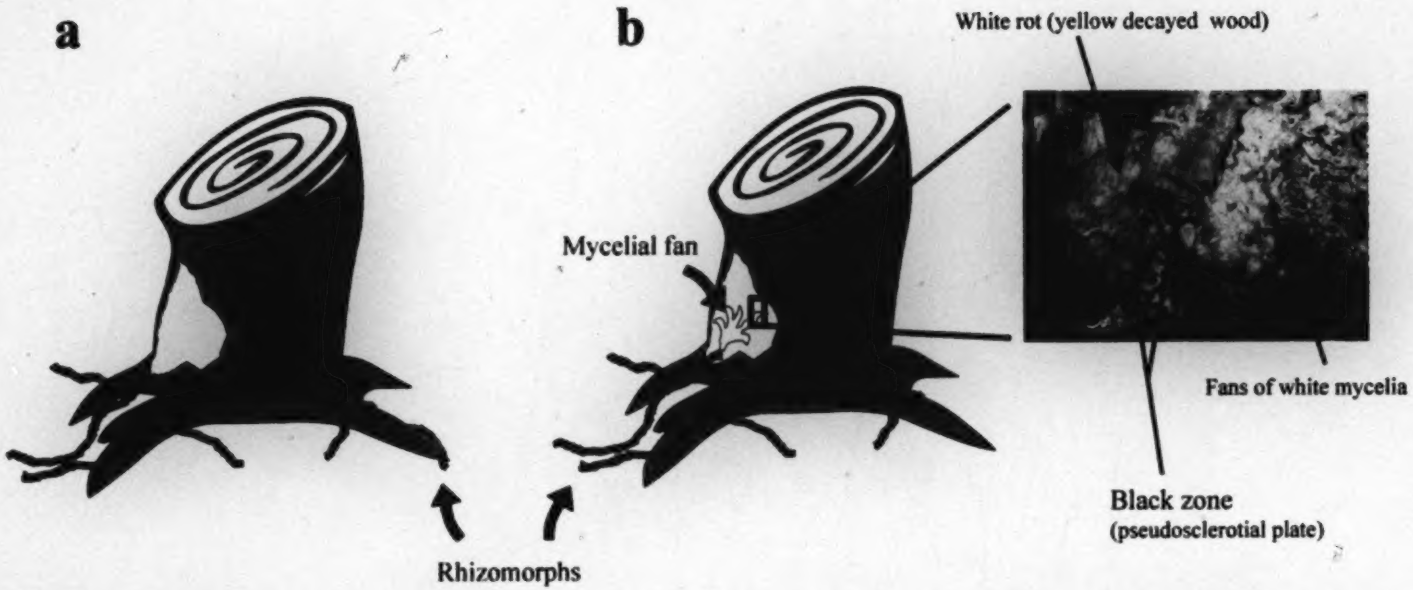
a
b
White rot (yellow decayed wood)
Mycelial fan
Fans of white mycelia
Black zone
(pseudosclerotial plate)
Rhizomorphs

Figure 1.2: Schematic of **a**) the presence but no infection by *Armillaria sinapina* rhizomorphs and **b**) infection by *A. sinapina* rhizomorphs. The yellow area on stump is a representation of the cambium after bark has been cut away. For both diagrams a rhizomorph is followed from the outside of the root collar to the inside of the root collar. In no infection diagram **a**) the rhizomorph has not penetrated the bark and into the cambium and in **b**) the rhizomorph has penetrated the outer bark and has started to grow into the cambium by forming a white mycelial fan. Over time melanized hyphae (pseudosclerotial plate) is formed and the *Armillaria* species causes yellow stringy rot termed white rot typical of *Armillaria* species.

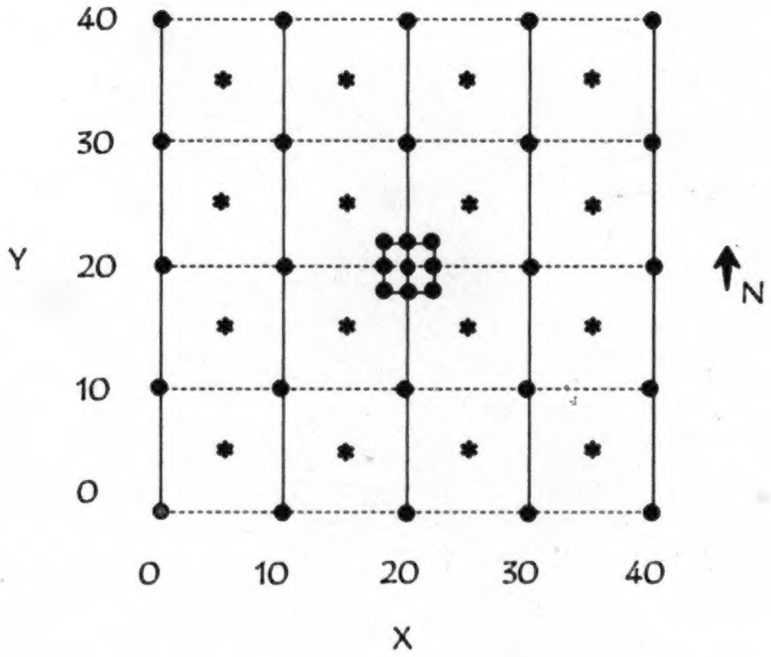

Figure 1.3: The field sampling layout. All trees, snags, and stumps were mapped in a 40 × 40m plot. The plot start position, shown as the grey dot (south-west corner), was permanently marked with a rebar stake. 33 aspen stakes (trap logs), shown as black dots; were staked on a 10m grid and one central 1 m grid for each plot. Downed woody material was sampled from west to east using the intercept method, on five 40m transects, shown as the dotted lines. 16 forest floor cores shown as stars, were sampled on a 10m grid for each plot. 16 resin bags were installed by the forest floor core and 16 forest floor moisture cores were taken by the forest floor cores.

Figure 1.4: Photograph of a gel showing some banding patterns of *Armillaria sinapina* using the *Alu*I restriction enzyme. The species identification of my isolates (Lanes 3-7) were determined by comparing the migration of the 'bands' base pairs (bp) distances of my isolates to those of the known *Armillaria* species (Lanes 1 and 2) and the DNA standard (Lane 8). Lane 1 shows the banding pattern 399-240-200-183-135 bp (pattern c) from known isolate *Armillaria sinapina* (NoF-894). Lane 2 shows the banding pattern 310-200-135 (pattern a) from known isolate *Armillaria ostoyae* (NoF-898). Lanes 3-6 (4 and 5 are very faint in this photo) show identified isolates from this current study to be *A. sinapina* with a banding pattern of 399-240-200-183-135 bp (pattern c). Lane 7 shows a banding pattern of 399-240-183 bp (pattern b) also identified as *A. sinapina*.

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Personal communication

Dr. Ken Mallett, 5320 122st, Northern Forestry Centre, Edmonton, T6H 3S5, Alberta, Canada

Dr. Alison Munson, Centre de recherche en biologie forestiere, Faculte de foresterie et de geomatique, Universite Laval, Sainte-Foy, G1P 3W8, Quebec, Canada

Colin Myrholm, 5320 122st, Northern Forestry Centre, Edmonton, T6H 3S5, Alberta, Canada

Chapter Two

Environmental predictors of *Armillaria sinapina* in trees, snags, stumps, downed woody material, and the forest floor in stands of boreal mixedwood forest

Introduction

Armillaria sinapina Bérubé and Dessureault is a basidiomycetous fungal species in the Canadian boreal forest (Morrison *et al.* 1985, Bérubé and Dessureault 1988, Dumas 1988, Bérubé 2000). This species potentially plays an important ecological role in the forest as both a pathogen and a saprophyte (Mallett 1990). Therefore, predicting its presence is of interest for both forest managers and forest ecologists alike. In order to be able to predict its presence, we must understand what environmental factors are important for its growth and survival. Environmental factors that have been shown to be important for the presence, growth, and pathogenicity of members of the genus *Armillaria*, including *A. sinapina* are: tree species, soil texture, soil temperature, soil pH, soil moisture, phosphorus concentration, ammonium (NH_4^+) concentration, organic matter content, and total basal area of stumps amongst others (e.g. Mallett 1990, Redfern and Philip 1991, Wiensczyk *et al.* 1997, Mallett and Maynard 1998).

To date, there have been no studies that have focused specifically on identifying the important environmental predictors of the occurrence of *Armillaria sinapina* on a small scale in Canadian boreal mixedwood forests. So far, all that has been established is that the species is readily baited from the soil with trap-logs and it has been stated that it prefers broadleaf over coniferous hosts (Mallett 1990, Blenis *et al.* 1995).

The purpose of the work described in this chapter was to identify the important environmental factors that are correlated with the occurrence of *Armillaria sinapina* in trees, snags, stumps, downed woody material (DWM), and the forest floor within nine randomly located 40 × 40 m plots, in boreal mixedwood forest stands in north-western Alberta. In addition, this chapter asks does *A. sinapina*, play a role in tree decline and death? The specific questions that were addressed are (1) What are the best environmental predictors of the presence of *A. sinapina* in trees, snags, and stumps, DWM, and the forest floor? (2) Is the health status of a tree (healthy, declining, dead) independent of its tree type (broadleaf, coniferous) and (3) Is the health status of a tree independent of infection by *A. sinapina*?

Methods

Data collection

Sampling took place at the EMEND (Ecological Management Emulating Natural Disturbance) field research site, located at township 89-90, range 03, west of the 6th meridian, 90km Northwest of Peace River, Alberta. A more detailed description of the study area is contained in Chapter One (page 12 and 13). *Armillaria sinapina* forms honey coloured basidiocarps in late fall and produces rhizomorphs prolifically via which it travels through the forest floor. Within nine 40 × 40 m plots, a total of 1,911 trees, snags, and stumps, 455 pieces of downed woody material (DWM), 144 forest floor cores, and 297 trap-logs were sampled for the presence of *Armillaria*.

Infected trees, snags, and stumps

Within each plot, all live trees, snags and stumps were mapped and categorized as coniferous or broadleaf and examined for the presence of *Armillaria*. In mid- June each tree was assigned to one of the following health categories: 1) healthy, 2) declining (i.e. visible outer signs of decline in broadleaf trees were yellowing and loss of leaves from the top part of the crown or half a crown present, visible outer signs of decline in coniferous trees were the loss of many needles, needles turning red, half the crown missing or copious amounts of resin seeping from the base of tree); 3) snag (i.e. rooted dead trees that were greater than 1.3 m but less than 10 m in height but this category also includes rooted dead trees that were greater than 10m tall); and 4) stumps (i.e. rooted dead trees that were less than 1.3 m in height). The roots and root collars of trees were inspected for *A. sinapina* infection (Chapter One, page 14). 'Infected' was where the rhizomorphs penetrated the outer bark and grew into a mycelial fan in the cambium area (Figures 1.1c and 1.2). Various properties of the trees, snags, and stumps themselves, which were thought to be potentially important for fungal growth were collected for all trees, snags and stumps (e.g. tree species and diameter at breast height) (Table 1.1).

Colonized downed woody material (DWM)

Downed woody material (DWM), greater than 7.5 cm in diameter, that was intercepted by the transect were inspected for the presence of *Armillaria* on five 40 m transects per plot (Figure 1.3). In addition, the bearing from the transect intercept to the base of the log (= direction of fall), the length from the transect intercept to the base, and the presence of *Armillaria* at the base, were recorded for those pieces of DWM with an intact base. The DWM was labelled as colonized if the fungus was present under the bark or inside the wood if the bark was missing, in the form of rhizomorphs, a melanized black zone-line, or a mycelial fan. The small number of pieces of DWM which were difficult to identify were assumed to be broadleaf DWM. Environmental variables, thought to be potentially important for fungal occurrence in DWM were recorded at each log intercept (e.g. DWM species and diameter) (Table 1.1).

Presence of rhizomorphs in the forest floor and colonizing trap-logs

Cores of the forest floor included the leaf litter layer, the fermenting layer and the humus layer (LFH). Within each of the nine plots, sixteen 10.3 cm diameter cores of the forest floor were taken on a 10 m grid (Figure 1.3). The depth of the forest floor core varied for each core because only the LFH layer was inspected for the presence of rhizomorphs in the lab. The volume of the LFH was calculated using $V = \pi r^2 h$. V equals the volume recorded as cm^3. π equals 3.14. r is the radius of the forest floor core which is 5.15cm and h is the depth of the forest floor core measured in centimetres. The length of rhizomorph pieces found within each core was also measured and a total rhizomorph length per volume of forest floor was calculated per forest floor core. These values were calculated by dividing the total length of the rhizomorphs pieces in centimetres by the volume of the LFH layer per forest floor core.

Several environmental variables associated with the forest floor were recorded (Table 1.1). A 5 cm diameter core of the forest floor was taken within 5 cm of the original core and used to measure soil moisture. In mid-May 1999, in each of the plots, sixteen ion-anionic exchange resin bags were placed between the bottom of the surface organic horizon (LFH) and the top mineral horizon on the 10 m grid. In mid-August 1999, 0.5 × 0.5 m vegetation quadrats were centred over each resin bag. The percent cover of each plant species was estimated using six cover classes (Table 1.1). The cover due to small woody debris (less than 1 cm in diameter), moss cover, and leaf litter was also estimated for each quadrat. For ease in the data analysis vegetation was grouped into four categories based on TWINSPAN analysis (a more detailed description is to follow):1) broadleaf wet 2) broadleaf dry 3) coniferous wet, and 4) coniferous dry. Cover of small woody debris and leaf litter was categorized as 1) absent 2) **little** (which includes cover estimates 1 and 2) 3) **moderate** (which includes cover estimates 3 and 4) or (4) **lots** (which includes cover estimates 5 and 6) (see Table 1.1 for cover classes).

In early May 1999, in each of the nine plots, thirty-three aspen trap-logs were installed on a 10 m grid and a 1 m grid in the plot centre (Figure 1.3). The trap-logs were removed in late August and were inspected for the presence of *Armillaria*. The trap-log was recorded as being colonized if rhizomorphs were growing along the bark and/or a mycelial fan was growing under the bark. As for the forest floor cores, 0.5 × 0.5 m vegetation quadrats were centred over each trap-log. The percent cover of each plant species was estimated using six cover classes (Table 1.1). The cover due to small woody debris (less than 1 cm in diameter) and leaf litter were also estimated for each quadrat. As described above discrete classes were used to quantify cover of vegetation, small woody debris and leaf litter for subsequent analyses.

Data analysis

All datasets were checked to see if they met the assumptions of normality and homoscedasticity prior to analysis. Where appropriate, data were transformed so that the assumptions of the various models used (e.g. parametric regression) were not violated. The transformations used were arcsine, square-root and log base 10.

To simplify the vegetation data for quadrats into a variable with four categories I used Two-way indicator species analysis (TWINSPAN) with PC-ORD version 4.0 (McCune and Mefford 1999). The first division split the broadleaf dominated plots from the coniferous dominated plots. The indicator species for the broadleaf dominated plots were *Pyrola asarifolia* Michx. found in moist woods (Moss 1983, Johnson *et al.* 1995), *Fragaria virginiana* Duchesne (found in dry to moist open woodlands) (Moss 1983, Johnson *et al.* 1995), and *Aster ciliolatus* Lindl. (found in open woodlands) (Moss 1983, Johnson *et al.* 1995). The indicator species for the coniferous dominated plots were moss species (common on forest floor of coniferous forests) and *Mitella nuda* L. (found in moist woods) (Moss 1983, Johnson *et al.* 1995). The second and third divisions by

TWINSPAN divided the broadleaf and coniferous categories into dryer and wetter vegetation types.

These four categories were groups of quadrats with similar species composition. Quadrats in group one (**broadleaf moist**) were categorized by the occurrence of the following plant species: *Petasites palmatus* (Ait.) A.Gray. found in moist woods and swamps (Moss 1983, Johnson *et al.* 1995), *Viburnum edule* (Michx.) Raf. found in moist woods and margins of wetlands and streambanks (Moss 1983, Johnson *et al.* 1995), *Fragaria virginiana* Duchesne found in dry to moist open woodlands (Moss 1983, Johnson *et al.* 1995).

Quadrats in group two (**broadleaf dry**) were categorized by the occurrence of *Elymus innovatus* Beal found in open woods, clearings, slopes, and moist meadows (Moss 1983, Johnson *et al.* 1995), *Lathyrus ochroleucus* Hook. found in open woods, and clearings (Moss 1983, Johnson *et al.* 1995), *Epilobium angustifolium* L. open woods, burned over areas and roadsides (Moss 1983, Johnson *et al.* 1995), *Galium boreale* L. found in open woods, clearings, meadows and roadsides (Moss 1983, Johnson *et al.* 1995) and *Rubus pubescens* Raf. found in moist woods and openings (Moss 1983, Johnson *et al.* 1995).

Group three (**coniferous moist**) were categorized by the occurrence of *Maianthemum canadense* Web.found in moist woods, often in sandy woods and clearings (Moss 1983, Johnson *et al.* 1995) and preferential species *Vaccinium caespitosum* Michx.woods and open slopes (Moss 1983, Johnson *et al.* 1995), *Vaccinium vitis-idaea* L. coniferous woods, moist forests, dry bogs and alpine slopes, and *Elymus innovatus* found in open woods, clearings, slopes, and moist meadows (Moss 1983, Johnson *et al.* 1995).

Group four (**coniferous dry**) were categorized by the occurrence of *Rosa acicularis* Lindl. found in open forests, clearings, riverbanks, and roadsides, *Lathyrus ochroleucus* found in open woods, and clearings, *Rubus pubescens* found in moist woods and openings, *Mitella nuda* found in moist woods (Moss 1983, Johnson *et al.* 1995).

To determine the best predictors of the presence of *Armillaria sinapina* on the sampled trees, snags, and stumps, DWM, trap-logs, and forest floor cores I used forward selection stepwise multiple logistic regression using generalised linear models assuming a binomial error distribution using S-Plus (version 6.2.1) (S-PLUS1988). Independent variables were selected for inclusion in the model based on a significant increase in residual deviance explained (F test). Only significant independent variables were included in the final model, the overall significance of which was then tested (F test; Sokal & Rohlf 1981). The following regressions were performed. Dependent variables (properties of the substrate plus environmental variables) were tested for their ability to predict: (1) the presence of *Armillaria sinapina* infecting trees, snags and stumps (Table 2.5), (2) the presence of *A. sinapina* colonizing under the bark of downed woody material (DWM) at the intercept (Table 2.7a) and at the base (Table 2.7b), and (3) the presence of *A. sinapina* infecting trap-logs (Table 2.9). Similarly, forward selection stepwise multiple regression using generalised linear models assuming a Gaussian error distribution was used to determine which independent variables were the best predictors of length of rhizomorphs per forest floor volume (Table 2.10). I also performed logistic regressions of the presence of *A. sinapina* colonizing DWM at the intercept versus (1) *A. sinapina* colonizing at the base and (2) the length between the intercept and the base point (Table 2.8). The environmental variables used in these analyses are shown in Chapter One, Table 1.1. To account for the structure of the data, the variable 'plot' was used as an independent (dummy) variable in these regressions. Still, for the regression analysis there is a heightened probability of Type I error because of the possibility of spatial autocorrelation among sampled substrates within plots (leading to non-independence of individual data points). Thus I treated all results where $P > 0.01$ with caution.

Contingency table analysis was used to examine the relationship between tree health, tree species, and infection by *Armillaria* as follows: (1) tree health (healthy, declining, dead) and tree type (broadleaf, coniferous) (Table 2.11), (2) broadleaf tree health and infection by *A. sinapina* (Table 2.12) and (3) coniferous tree health and infection by *A. sinapina* (Table 2.13). Testing was by means of the Chi-square statistic for goodness of fit (or Pearson's chi-square, Sokal and Rohlf 1981) using S-Plus (version 6.2.1) (S-PLUS 1988). If there were significant results in the first (2 X 3) analysis subsequent analyses were performed on reduced (2 x 2) contingency tables to further elucidate where the significant differences lay (Sokal and Rohlf 1981).

Results

The total number of trees, snags, and stumps encountered in each of the nine plots ranged from 134 to 314 for a total of 1911 (Table 2.1). The nine plots represented a wide range in terms of broadleaf proportion (7.2% to 92.0% broadleaf for trees, snags, and stumps; Table 2.1). In the two plots with 90% and 92% broadleaf trees, snags, and stumps, the coniferous trees were in the understory. The proportion of infected trees ranged from 16% to 92% (Table 2.1). Most of the broadleaf trees, snags, and stumps were infected by *Armillaria sinapina* in every plot. The total proportion of sampled broadleaf trees, snags and stumps infected per plot ranged from 60 to 98% (Table 2.1). Conversely, coniferous trees, snags, and stumps were not heavily infected by *A. sinapina*. The infection ranged from 0 to 27% (Table 2.1).

The number of pieces of downed woody material (DWM) sampled at the intercept and at the base in the nine plots ranged from 19 to 85 and 12 to 69, respectively (Table 2.2). The proportion of pieces of DWM that was colonized at the intercept in the nine plots ranged from 8% to 61% (Table 2.2). The proportion of DWM pieces that were colonized at the base in the nine plots ranged from 20% to 71% (Table 2.2). For each of the nine plots a higher proportion of pieces of DWM sampled at the base were colonized than the intercept (Table 2.2). Sixteen forest floor cores and 33 trap-logs were successfully obtained from each of the 9 plots. Rhizomorphs were found in the forest floor cores and were colonizing the trap-logs in all nine plots (Table 2.3). Between 31 and 100% of the 16 forest floor cores contained rhizomorphs (Table 2.3). Between 18 and 91% of trap-logs were colonized in each of the nine plots (Table 2.3).

Regression to predict presence of Armillaria *on trees, snags, and stumps*

The total number of trees, snags, and stumps sampled were 1911. Broadleaf trees, snags, and stumps numbered 943 and coniferous trees, snags, and stumps numbered 968 (Table 2.4). 90% of the broadleaf trees, snags, and stumps were infected by *Armillaria sinapina*

(Table 2.4). In contrast, only 14% of the coniferous trees, snags, and stumps were infected by *A. sinapina* (Table 2.4).

All plots were combined for the regression analysis. Species (broadleaf versus coniferous) of tree, snag or stump explained 47.6% of the variation in the presence of *Armillaria sinapina* infecting trees, snags, and stumps (Table 2.5). Broadleaf trees were more likely to be infected than coniferous trees. The dummy variable 'plot' was highly significant but only explained an additional 1.9% of the variation. Tree height entered the model and was negatively related to the presence of *A. sinapina*, but explained very little of the variation (0.2%).

Regression to predict presence of Armillaria *on downed woody material*

The total number of pieces of downed woody material (DWM) sampled at the intercept was 455 (Table 2.6). 81% of the pieces were broadleaf DWM and of those pieces 39% were colonized by *Armillaria sinapina* (Table 2.6). 19% of the pieces were coniferous DWM and of those pieces only 11% were colonized by *A. sinapina* (Table 2.6).

All plots were combined for the regression analysis. Logistic regression showed that the best predictor of *Armillaria sinapina* colonizing at the intercept was whether the DWM was on or off the ground (Table 2.7). When DWM was on the ground it was more likely to be colonized by *A. sinapina* (this variable explained 15.6% of the deviance in the model) (Table 2.7). The dummy variable 'plot' was significant and explained 8.5% of the deviance. DWM species explained 4.9% of the deviance; broadleaf logs were more likely to be colonized than coniferous (Table 2.7). Also, larger pieces of DWM were more likely to be colonized and this variable explained 2.1% of the deviance (Table 2.7).

The total number of pieces of DWM sampled at the base was 327 (Table 2.6). 79% of the pieces were broadleaf DWM and of those pieces 61% were colonized by *A. sinapina* (Table 2.6). 21% of the pieces were coniferous DWM and of those pieces 20% were colonized by *A. sinapina* (Table 2.6). The best predictor of *Armillaria sinapina* colonizing at the base was DWM species. Broadleaf DWM were more likely to be colonized than coniferous DWM and this explained 8.7% of the deviance (Table 2.7). The dummy variable 'plot' and DWM on or off the ground also entered the model (Table 2.7). Pieces of DWM on the ground were more likely to be colonized by *A. sinapina*. The direction of DWM fall entered the model but explained only 1.7% of the deviance.

The sampled piece of DWM that had intact bases were used to test if the presence of *A. sinapina* colonizing at the base could predict the presence of *A. sinapina* colonizing at the intercept (Table 2.8). The model explained 18.6% of the deviance in presence of *A. sinapina* colonizing DWM with intact bases. The closer the intercept point was to a colonized base point the more likely it was that the intercept point was colonized (Table 2.8).

Regression to predict presence of Armillaria *in the forest floor and trap-logs*

Of the 297 trap-logs installed in this study 38% were infected by *Armillaria sinapina* (Table 2.3). Of the 144 soil cores sampled 58% showed the presence of *A. sinapina* rhizomorphs (Table 2.3).

Multiple logistic regression showed that the cover of small woody debris, litter cover and vegetation type explained 14% of the deviance in the presence of *Armillaria sinapina* colonizing trap-logs (Table 2.9). The vegetation categories "broadleaf moist" and "broadleaf dry" had a higher probability of colonization of trap-logs. The more leaf litter there was the higher the probability of colonization of trap-logs. Even though small woody debris entered the model the probability of colonization of trap-logs appeared to be similar for all categories.

Multiple linear regression showed that the dummy variable 'plot' explained the most deviance (23.6%) in the length of rhizomorphs of forest floor volume (Table 2.10). Soil nutrients were correlated with the length of rhizomorphs per forest floor volume: calcium was negatively correlated (3.2% deviance explained), sulphur was negatively correlated (3.4% deviance explained), and potassium was positively correlated (1.9 % deviance explained). Depth of the forest floor was also negatively correlated with the length of the rhizomorphs per forest floor volume and explained (6.9%) of the deviance (Table 2.10).

Trends for tree health

A qualitative examination of the data suggests that as the proportion of broadleaf trees, snags, and stumps per plot increased, so did the proportion of infected trees snags and stumps (Figure 2.1). Furthermore, as the proportion of broadleaf trees increased the proportion of healthy trees decreased (Figure 2.1). The contingency analysis detected some differences among tree types (broadleaf, coniferous) in the observed *vs* expected frequencies in different health categories (healthy, declining, dead) (Pearson's chi-square $\chi^2 = 214.2$, df = 2, $P<0.0001$) (Table 2.11, Figure 2.2). Given that there was a difference where was it arising? Because there were relatively few trees in the "declining trees" category, and the question was about the effect on health of trees, I chose to group declining and dead trees into one category and then used a 2 x 2 contingency analysis to compare broadleaf vs coniferous trees in terms of the frequency that were declining/dead versus healthy. The results showed that a significantly higher proportion of coniferous trees was healthy (78% versus only 46% of broadleaf trees) (Pearson's chi-square $\chi^2 = 209.9$ df = 1, $P<0.0001$) (Table 2.11, Figure 2.2).

For broadleaf trees there was no difference between those infected by *Armillaria sinapina* and those not infected in terms of the frequency in different health categories (Pearson's chi-square $\chi^2 = 3.99$, df = 2, $P = 0.136$) (Table 2.12, Figure 2.2). However, for coniferous trees there was a difference (Pearson's chi-square test $\chi^2 = 35.8$, df = 2, $P<0.0001$) (Table 2.13, Figure 2.2). Because there were relatively few declining trees and the question was about the effect of infection on mortality I grouped healthy and declining trees into one category and then used a 2 x 2 contingency analysis to compare infected vs uninfected coniferous trees in terms of the frequency that were alive (healthy or declining) vs dead. The results showed that significantly higher proportion of the infected (vs uninfected) coniferous trees were dead (26% versus only 13% of the uninfected trees) (Pearson's chi-square $\chi^2 = 23.9$, df = 1, $P<0.0001$) (Table 2.13, Figure 2.2).

Discussion

The analyses in this chapter identify the important predictors for the presence of *Armillaria sinapina* infecting trees, snags, and stumps and occurring in downed woody material (DWM), forest floor cores, and trap-logs. These results allow some comment to be made on the relationships between tree health and the presence of *Armillaria sinapina*.

Trends for trees, snags, and stumps (tss)

This study was somewhat limited in its ability to determine the best predictors of the presence of *Armillaria sinapina* because of the small sample size (nine plots). However, there are some generalisations and hypotheses that can be made from the patterns arising from my results. It was clear that tree species was the most important determinant of the presence of *A. sinapina* on live trees or on snags or stumps. A higher proportion of sampled broadleaf trees, snags and stumps were infected; also, as the proportion of broadleaf trees, snags, and stumps per plot increased, so did the proportion of infected trees snags and stumps. However there was no relationship between tree composition of the plots and the probability of infection for either broadleaf or conifers. In the broadleaf-dominated plots, which had overall high infection rates, infection rates for conifers were just as low and for broadleaves were just as high as in conifer-dominated plots, which overall had low infection rates (Table 2.1). This suggests that *A. sinapina* prefers broadleaf substrates over coniferous, supporting similar conclusions made by other researchers using different methods (Mallett 1990, Blenis *et al.* 1995). It also suggests that the probabilities of infection for either broadleaf or coniferous substrates are not related to other site factors or forest structural or successional factors.

Trends for downed woody material (DWM)

As for trees, snags, and stumps, broadleaf DWM was more likely to be colonized by *Armillaria sinapina* at the base and "broadleaf" vs "conifer" explained the greatest proportion of variation in that regression. In contrast, "broadleaf" vs "conifer" explained a relatively small amount of the variation in probability of infection at the intercept. Instead, the most important factor explaining the probability of infection at the intercept was whether the DWM was on or off the ground. Whether DWM was on or off the ground was the second most important factor explaining probability of infection at the base. DWM that was on the ground was more likely to be colonized by *Armillaria sinapina* at both the base and the intercept than DWM that was not in contact with the ground. As downed log decay progresses moisture content increases 5- to 10-fold from early stages of decay to advanced stages of decay (Jurgensen *et al.* 1984, Larsen *et al.* 1982). It is likely that DWM that was on the ground was moister, softer and easier for *A. sinapina* rhizomorphs to penetrate and grow through. This may explain why *A. sinapina* was more likely to be found at both the intercept and base in DWM on the ground. Also, when DWM is in contact with the ground there is a chance for it to be encountered by rhizomorphs in the forest floor. When a piece of DWM is not on the ground it is less likely to be colonized at the intercept because the rhizomorphs would not encounter it. Larger pieces of DWM (larger intercepted diameters) were also somewhat more likely to be colonized, potentially because larger pieces are more likely to be encountered by the fungus by chance, larger pieces are more likely to be lying on the ground rather than hung up in another tree, and larger-diameter intercepts are likely closer to the base (see below).

Every piece of DWM with an intact base was examined for the presence of *A. sinapina* at the intercept and at the base. Intercepts that were closer to an infected base were more likely to be infected than intercepts that were further away. This suggests that if *A. sinapina* is present at the base when the DWM is a standing tree the fungi can spread from the base to colonise the intercept. Together, these results suggest that colonization of DWM by *A. sinapina* occurs both by means of expansion of a basal infection, which existed in the live tree, and by new colonization of downed logs as they are contacted by rhizomorphs moving through the LFH layer. It can be hypothesized that *A. sinapina* may have played a role in causing tree death and creating DWM. However, it is noteworthy that for broadleaf substrates the proportion of DWM infected at the base (0.61) was lower than the proportion of broadleaf trees, snags and stumps infected while for conifer substrates the proportion of DWM infected was somewhat higher than the proportion infection for trees, snags and stumps.

Trends for rhizomorphs in the forest floor and presence of Armillaria sinapina *colonizing trap-logs*

Some studies have linked soil properties such as texture, nutrients, pH and moisture with the incidence and severity of *Armillaria* root disease (Redfern and Filip 1991, Wargo and Harrington 1991, Mallett and Maynard 1998). This study did not attempt to correlate forest floor nutrients with the presence of *A. sinapina* infection in trees, snags, and stumps but it did address the effect of the forest floor layer itself (nutrients, moisture) on the presence of *A. sinapina* rhizomorphs. As with some previous studies the greatest concentration of rhizomorphs was found in the humus layer of the forest floor samples (Hintikka 1974, Singh 1981). The presence of *A. sinapina* rhizomorphs was negatively related to the soil nutrients calcium and sulphur and the depth of the organic layer and positively related to potassium. However, none of these nutrients explained much of the variation in abundance of rhizomorphs in the forest floor and the other forest floor nutrients and moisture were unimportant in explaining the presence of *A. sinapina* rhizomorphs. In addition, laboratory experiments and field observations have shown that *Armillaria* rhizomorphs can grow in a wide range of forest and agricultural soils (Morrison 1976, Redfern 1973, Rishbeth 1985, Redfern and Filip 1991). This may be because they are simply travelling through this substrate to 'find' more suitable substrates. However, it is quite possible that the appropriate forest floor characteristics were not measured in this study.

There was some suggestion from the trap-log data that the immediately surrounding vegetation had an influence on the amount of *A. sinapina* rhizomorphs. Colonization of trap-logs was greater in locations surrounded by wet broadleaf vegetation and less in samples that were surrounded by dry and coniferous vegetation. This may simply be a reflection of the preference of *A. sinapina* for broadleaf species over coniferous (Mallett 1990) such that rhizomorph abundance is higher in stands which are dominated by the preferred host. This is also supported by the fact that "plot" explained the greatest amount of variation in rhizomorph abundance in forest floor samples.

Overall, these results suggest that the *A. sinapina* rhizomorphs are capable of existing in a range of soil/forest floor conditions and are likely just travelling through it in search of new, uncolonized substrates.

The trap-log method (Mallett and Hiratsuka 1985) may be a good non-invasive method of sampling that allows us to predict the occurrence of *Armillaria sinapina* in the surrounding forest trees. This study did not address this question and therefore further exploration is needed.

Tree health and Armillaria sinapina

If *Armillaria sinapina* was causing disease and death in the trees in the plots considered in this study, declining trees, snags, and stumps should have shown a high percentage of infection by *A. sinapina* than healthy trees. However, this was only partially true. Tree health was confounded with the variable 'species': more coniferous trees were healthy than broadleaf and *A. sinapina* appeared to prefer to infect broadleaf over coniferous, so an appearance that infection correlated strongly with tree health was in fact simply due to tree species. When healthy, declining, and dead trees were compared within broadleaf, no health category was more or less likely to be infected. Also recall that the proportional basal infection of broadleaf DWM was lower than infection of broadleaf trees, snags and stumps. From this study, it is difficult to conclude that *A. sinapina* is playing a major role in causing broadleaf death because healthy and dead broadleaves were equally infected. Although it is clear that *A. sinapina* definitely prefers to colonize broadleaf hosts further study is needed to assess whether an infection by *A. sinapina* in a healthy broadleaf tree ultimately leads to the death of the tree. It appears that *A. sinapina* is a common saprophyte of broadleaf trees, snags, stumps and downed woody material and at times could change roles to be a pathogen of broadleaf trees. This ability to switch roles from a saprophyte to a pathogen could be tested in a laboratory situation.

For conifers, however, my results suggest that *A. sinapina* may be playing a role in the tree decline or death. When healthy and dead coniferous trees were compared dead coniferous trees were more likely to be infected by *A. sinapina*. Further, recall that the proportion of conifer DWM pieces that were colonized at the base was higher than the proportional colonization of trees, snags, and stumps. In boreal forests of North America, *A. sinapina* is thought to be less pathogenic than *A. ostoyae* especially in regards to conifer infection and disease (Morrison et al 1985, Dumas 1988, Kile et al 1991). In a laboratory experiment, white spruce and lodgepole pine seedlings were infected by *A. sinapina* but *A. sinapina* isolates did not cause mortality (Mugala *et al*. 1989). In contrast, a laboratory study showed that lodgepole pine seedlings inoculated with *A. ostoyae* and *A. sinapina* were more susceptible to infection and death by *A. sinapina* (Mallett and Hiratsuka 1988). In addition, Mallett (pers. comm., unpublished data) has repeated these experiments several times and has found *A. sinapina* causing mortality in white spruce. In this study, *A. sinapina* appears to be playing a role in conifer tree death.

Summary and conclusions

This research is unique in that this is the first time that the ecology and occurrence of *Armillaria sinapina* has been studied in such detail on a small scale. An important result was that *A. sinapina* preferred to infect and colonize broadleaf hosts in all the plots regardless of tree health. Conversely, coniferous trees were less likely to be infected by *A. sinapina*. *A. sinapina* was more likely to be found in broadleaf pieces of DWM with larger diameters and that were lying on the ground. In addition *A. sinapina* was present in a higher proportion in broadleaf DWM sampled at the base. This may suggest that the fungus was present in the living tree before it became DWM. *A. sinapina* occurred in the forest floor in the form of rhizomorphs and its presence was partially dependent on some soil nutrients (K, Ca, and S) and the depth of the organic layer. The fungus was easily baited from the forest floor using trap-logs. These results suggest that the fungus uses the forest floor primarily as a travelling medium for vegetative dispersal to new woody substrates. This study shows that *A. sinapina* is not obviously a major cause of tree death in the broadleaf trees and it is likely living as a saprophyte in broadleaf substrates but further study is needed to understand its role as a saprophyte and possibly as a pathogen in broadleaf hosts. However, my results support the suggestion that *A. sinapina* may be causing death in coniferous trees, albeit in a very low proportion.

Table 2.1: A summary table showing for each plot the number of healthy trees, number of declining trees, number of snags and stumps and the total number of trees, snags and stumps (tss). The proportion of trees, snags and stumps that were broadleaf, the proportion of trees, snags, and stumps that were infected, and the proportion of broadleaf and coniferous trees, snags, and stumps that were infected are also reported

Plot	# healthy trees	# declining trees	# snags and stumps	Total tss	Proportion broadleaf tss	Proportion infected tss	Proportion broadleaf tss infected	Proportion of coniferous tss infected
1	119	8	26	153	0.07	0.16	0.82	0.11
2	130	4	45	179	0.19	0.25	0.59	0.17
3	132	2	64	198	0.20	0.32	0.90	0.18
4	165	34	76	275	0.30	0.36	0.86	0.15
5	89	5	49	143	0.37	0.51	0.92	0.27
6	79	7	48	134	0.37	0.37	0.96	0.02
7	181	13	84	278	0.62	0.52	0.79	0.08
8	139	8	90	237	0.90	0.89	0.98	0.04
9	153	35	126	314	0.92	0.86	0.93	0.00

Table 2.2: A summary table showing for each plot the number of downed woody material pieces sampled at the intercept and at the base. The proportion of DWM pieces that were colonized at the intercept and the base.

Plot	# DWM pieces intercept	Proportion of DWM colonised intercept	# DWM pieces base	Proportion of DWM colonised base
1	19	0.32	12	0.50
2	52	0.08	45	0.20
3	44	0.48	32	0.59
4	36	0.61	19	0.63
5	47	0.40	22	0.54
6	36	0.58	23	0.70
7	64	0.30	60	0.42
8	70	0.39	45	0.71
9	85	0.16	69	0.58

Table 2.3: A summary table for each plot showing the number of trap-logs infected and the number of forest floor cores in which rhizomorphs were present. The proportions of infected trap-logs and proportion of forest floor cores with rhizomorphs of *A. sinapina* are also shown. The final row shows the total for all plots combined.

Plot	# trap-logs infected (out of 33)	Proportion of trap-logs infected	# forest floor cores with rhizomorphs (out of 16)	Proportion of forest floor cores with rhizomorphs
1	9	0.27	7	0.44
2	11	0.33	7	0.44
3	6	0.18	5	0.31
4	14	0.42	16	1.00
5	7	0.21	12	0.75
6	6	0.18	6	0.38
7	16	0.48	8	0.50
8	30	0.91	16	1.00
9	15	0.45	6	0.38
Total	**114**	**0.38**	**83**	**0.58**

Table 2.4: Summary tables for all nine plots combined of *a)* broadleaf; and *b)* coniferous trees, snags, and stumps (tss) sampled for the presence of *Armillaria*. Reported are the proportion of total sampled tss which were broadleaf and coniferous and, for each separately, the proportions of sampled tss which were: healthy trees, declining trees, dead snags and stumps (ss) along with the proportion of all sampled tss which were infected and, of those, the proportion that were: healthy trees, declining trees, and dead ss. In total 1911 trees snags and stumps were sampled.

a) Broadleaf trees sampled for the presence of *Armillaria*.

Total Number of Trees	Broadleaf trees	Healthy	Declining	Dead	Infected tss	Infected and healthy	Infected and declining	Infected and dead ss
1911	0.49 *n*=943	0.46 *n*=431	0.07 *n*=69	0.47 *n*=443	0.90 *n*=847	0.45 *n*=379	0.07 *n*=64	0.48 *n*=404

b) Coniferous trees sampled for the presence of *Armillaria*.

Total Number of Trees	Coniferous trees	Healthy	Declining	Dead	Infected tss	Infected and healthy	Infected and declining	Infected and dead ss
1911	0.51 *n*=968	0.78 *n*=756	0.04 *n*=47	0.18 *n*=165	0.14 *n*=132	0.30 *n*=39	0.02 *n*=3	0.68 *n*=90

Table 2.5: Results from forward multiple stepwise logistic regressions using generalized linear models (assuming a binomial error distribution) of the presence of *Armillaria sinapina* infecting trees, snags and stumps versus the measured environmental variables. N is the total number of trees, snags and stumps. The independent variables tested for inclusion in the model were, tree species, tree basal diameter, tree height and plot. The categorical variables, tree species and plot, were treated as dummy variables in this analysis.

N	Predictor	R^2	sign	*P*
1911	Tree species	0.476	-	<0.0001
	Plot	0.019		<0.0001
	Tree height (m)	0.002	-	0.0210
	Total =	**0.497**		

Table 2.6: Summary tables of the number of downed woody material (DWM) pieces that were sampled at the a) intercept and at the b) base. For each I report the number and proportion of pieces that were broadleaf and coniferous and for each of these the proportion of pieces that were colonized by *Armillaria*.

a) Downed woody material sampled at the *intercept*

# DWM pieces Intercept	Proportion of Broadleaf DWM	Proportion of Coniferous DWM	Proportion of Broadleaf DWM colonised	Proportion of Coniferous DWM colonised
455	0.813 *n*=370	0.187 *n*=85	0.389 *n*=144	0.105 n=9

b) Downed woody material sampled at the *base*

# DWM pieces Base	Proportion of Broadleaf DWM	Proportion of Coniferous DWM	Proportion of Broadleaf DWM colonised	Proportion of Coniferous DWM colonised
327	0.786 *n*=257	0.214 *n*=70	0.611 *n*=157	0.200 *n*=14

Table 2.7: Results from forward stepwise logistic regressions using generalized linear models (assuming a binomial error distribution) to predict the presence of *Armillaria sinapina* colonising downed woody material (DWM) at the a) intercept and b) base as a function of the measured environmental variables. N is the total number of pieces of DWM. The independent variables which were tested for inclusion in the model were: DWM species (0 = broadleaf vs 1 = coniferous, DWM on (0) or off (1) the ground, diameter of DWM at line intercept and (for b) only) the direction of fall of DWM. The categorical variables, plot, DWM species, DWM on or off the ground, and direction of tree fall were treated as dummy variables in this analysis. The sign of the coefficient is not given for factors with greater than two levels.

a) *intercept*

N	Predictor	R^2	sign	*P*
455	DWM on or off the ground	0.156	-	<0.0001
	Plot	0.085		<0.0001
	DWM species	0.049	-	<0.0001
	Diameter of DWM at line intercept	0.021	+	0.00051
	Total =	**0.311**		

b) *base*

N	Predictor	R^2	sign	*P*
327	DWM species	0.087	-	<0.0001
	Direction of tree fall	0.017		0.049
	Plot	0.071		<0.0001
	DWM on or off the ground	0.061	-	<0.0001
	Total =	**0.236**		

Table 2.8: Results from a forward stepwise logistic regression (assuming a binomial error distribution) predicting the presence of *Armillaria sinapina* colonising the intercept of a piece of downed woody material (DWM) as a function of: the presence of *A. sinapina* colonising the base of that piece of DWM and the length between the intercept and base. Presence at the base was treated as a dummy variable (0 = absent, 1 = present). Plot was included in the analysis but it was not significant. Both independent variables tesed for inclusion in the model were significant at $P<0.05$ and were included in the final model. + and - represents sign of the coefficient.

N	Predictor	R^2	sign	*P*
327	Presence at base	0.171	+	<0.0001
	Length between intercept and base point	0.015	-	0.0167
	Total =	**0.186**		

Table 2.9: Results from forward stepwise logistic regression using generalized linear models (assuming a binomial error distribution) to predict the presence of *Armillaria sinapina* infecting trap-logs as a function of the measured environmental variables. N is the total number of trap-logs. The independent variables tested for inclusion in the model were: vegetation type (which of the four TWINSPAN categories), leaf litter cover, and small woody debris cover. Since each of these were in classes they were treated as dummy variables in this analysis. Plot was treated as a dummy variable but was not significant so was not included in the final model. The sign of the coefficient is not given for factors with greater than two levels.

N	Predictor	R^2	sign	*P*
297	Small woody debris	0.028		0.0046
	Leaf litter	0.030		0.0173
	Vegetation type	0.078		<0.0001
	Total =	**0.140**		

Table 2.10: Results from forward stepwise multiple linear regression predicting the length of *Armillaria sinapina* rhizomorphs in forest floor cores as a function of the measured environmental variables. The independent variables tested for inclusion in the model were: plot, Ca, Mg, Mn, K, P, S, NH_4, NO_3, moisture, depth of the organic layer, vegetation type (which of the four TWINSPAN categories), leaf litter cover, and small woody debris cover. The categorical variables; vegetation cover, leaf litter cover, small woody debris cover, and plot were treated as dummy variables in this analysis. The sign of the coefficient is not given for plot because it is not meaningful.

N	Predictor	R^2	sign	*P*
144	Ca	0.032	-	0.009
	K	0.019	+	0.037
	S	0.034	-	0.007
	Depth of the organic layer	0.069	-	0.0002
	Plot	0.236		<0.0001
	Total =	**0.389**		

Table 2.11: Results from a 2 × 3 contingency table analysis for all 1911 trees, snags and stumps sampled comparing tree health (healthy, declining, dead) versus tree species (broadleaf, coniferous) *(a)*. Since a difference was detected in this analysis I combined declining trees and the dead trees before undertaking a 2 × 2 contingency analysis *(b)* comparing tree health (healthy, decline/dead) versus tree species (broadleaf, coniferous). The proportions and counts are reported. Results are presented as χ^2 –values (Pearson's chi-square statistic), degrees of freedom and with a *P*-value.

a) 2 × 3 contingency table comparing tree health and tree species

Tree type	Healthy	Decline	Dead
Broadleaf	0.458 *n*=431	0.072 *n*=68	0.470 *n*=443
Coniferous	0.780 *n*=756	0.041 *n*=40	0.179 *n*=173

χ^2= 214.2 df = 2 $P < 0.0001$

b) 2 × 2 contingency table comparing tree health versus tree species

Tree type	Healthy	Decline/Dead
Broadleaf	0.458 *n*=431	0.542 *n*=511
Coniferous	0.780 *n*=756	0.220 *n*=213

χ^2= 209.9 df = 1 $P < 0.0001$

Table 2.12: Results from a 2 × 3 contingency analysis comparing broadleaf tree health (healthy, declining, dead) and infection by *Armillaria sinapina*. The proportions and counts are reported. Results are presented as χ^2 –values (Pearson's chi-square statistic), degrees of freedom and with a *P*-value.

Infection	Healthy	Decline	Dead
Infected	0.45 *n*=379	0.08 *n*=64	0.48 *n*=404
Not infected	0.55 *n*=52	0.04 *n*=4	0.41 *n*=39

$\chi^2 = 3.99$ df = 2 $P = 0.136$

Table 2.13: Results from a 2 × 3 contingency analysis comparing coniferous tree health (healthy, declining, dead) versus infection by *Armillaria sinapina (a)*. Given that a difference was detected healthy trees and declining trees were combined before undertaking a 2 × 2 contingency analysis *(b)* comparing coniferous tree health (alive, dead) versus infection. The proportions and counts are reported. Results are presented as χ^2 –values (Pearson's chi-square statistic), degrees of freedom and P-value.

a) 2 × 3 contingency table comparing coniferous tree health and infection

Infection	Healthy	Decline	Dead
Infected	0.73 n=252	0.01 n=3	0.26 n=90
Not infected	0.81 n=504	0.06 n=37	0.13 n=83

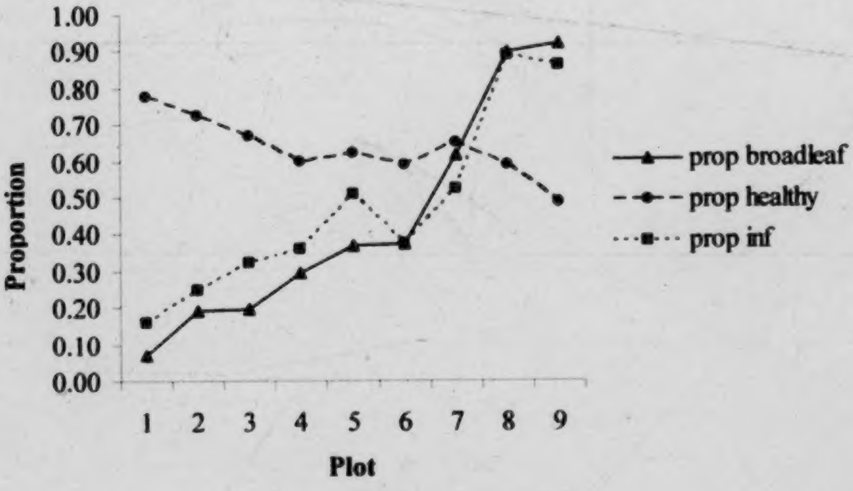
χ^2= 35.8 df = 2 $P < 0.0001$

b) 2 × 2 contingency table comparing coniferous tree health (Alive and Dead) and infection

Infection	Alive	Dead
Infected	0.74 n=255	0.26 n=90
Not infected	0.87 n=541	0.13 n=83

χ^2= 23.9 df = 1 $P < 0.0001$

Figure 2.1: A line graph showing the proportion of all sampled trees, snags, and stumps that were broadleaf (prop broadleaf), the proportion of trees, snags, and stumps that were infected (prop inf) and the proportion of healthy trees (prop healthy) for each plot ordered by increasing percentage of broadleaf trees, snags, and stumps.



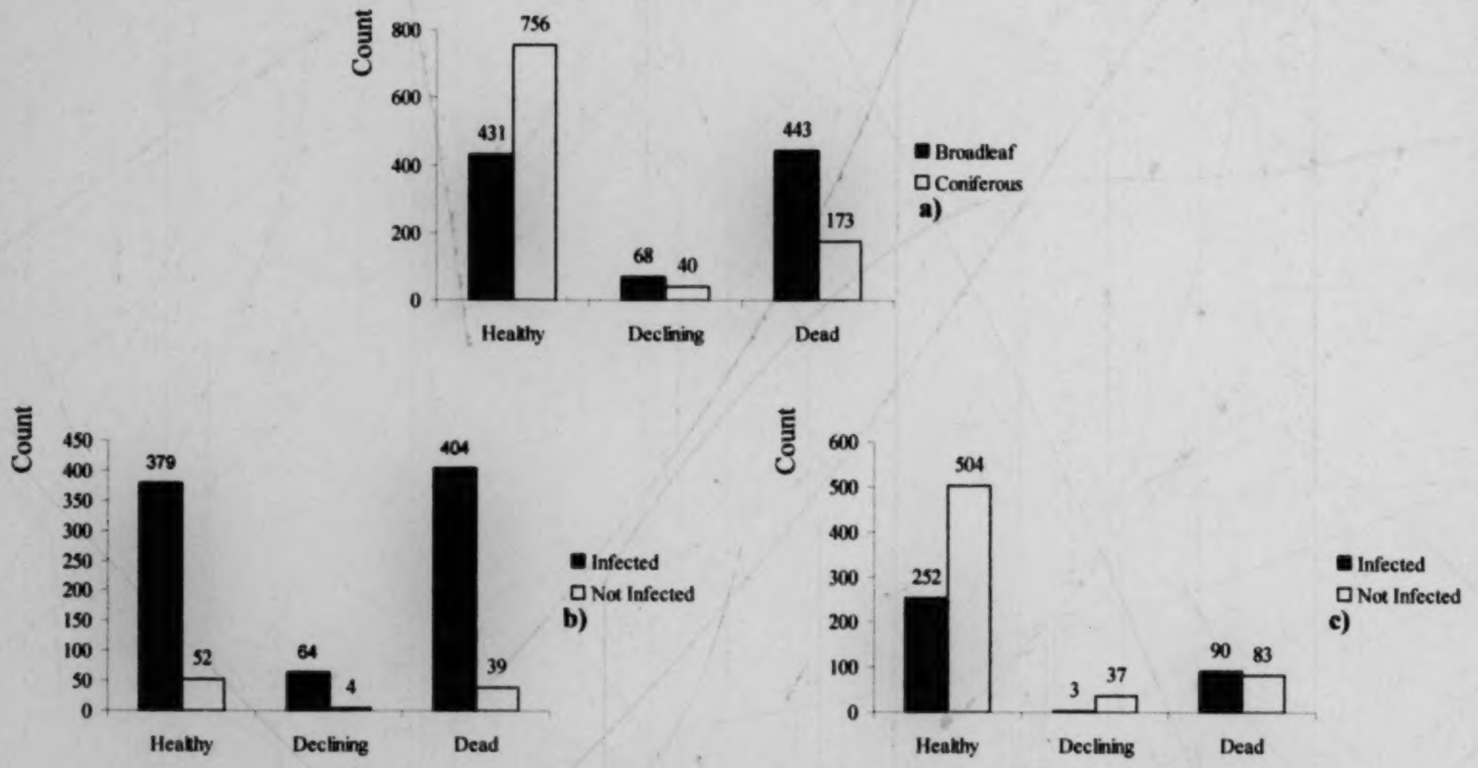


Figure 2.2: **a**) A bar graph showing the number of healthy trees, declining trees, and dead snags and stumps for broadleaf (black bars) and coniferous (white bars), for all the plots combined. **b**) A bar graph showing the number of broadleaf healthy trees, declining trees, and dead snags and stumps that were infected (black bars) or not (white bars). **c**) A bar graph showing the number of healthy coniferous trees, declining trees, and dead snags and stumps that were infected (black bars) or not (white bars).

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Personal Communication

Dr. Ken Mallett, 5320 122st, Northern Forestry Centre, Edmonton, T6H 3S5, Alberta, Canada

Chapter Three

Spatial pattern of *Armillaria sinapina* in stands of boreal mixed-wood forest

Introduction

Understanding the way in which a species is distributed in space, can reveal much about its ecology and allow predictions to be made about its response to changes in the biotic and abiotic environments in which it occurs (Dale 1999). The distribution of a pathogen is dependent on three sets of factors (1) abiotic, such as moisture and nutrient levels; (2) biotic, such as the distribution of competitors; and (3) the distribution and ecology of the host species.

There are two known species of *Armillaria* in Alberta, *Armillaria sinapina* and *Armillaria ostoyae* (Mallett 1990). *Armillaria sinapina*, the subject of this chapter, is a fungal pathogen that also acts saprophytically In the boreal mixedwood forest of north-western Alberta, it is found infecting a range of host trees including both broadleaf and coniferous species (Mallett 1990, Chapter 2). The two most common host trees within the study area were *Populus tremuloides* (aspen) and *Picea glauca* (white spruce). Although not yet empirically demonstrated in the literature (see Chapter Two), *A. sinapina* has been reported as being preferentially associated with broadleaf hosts and less frequently with coniferous hosts throughout Canada and the U.S.A. (Morrison *et al.* 1985, Bérubé and Dessureault 1988, Dumas 1988, Mallett 1990, Blodgett 1992, Banik *et al.* 1995, Frontz *et al.* 1997, Bérubé 2000). This chapter specifically addresses the spatial pattern of *A. sinapina* on a small scale (within nine 40 x 40m plots).

Spatial pattern of Armillaria

Relatively little is known about the spatial pattern of *Armillaria sinapina*. No studies have been done that explicitly described the spatial pattern, if any, of *A. sinapina* in the boreal mixedwood forest or investigated the possible underlying causes. However, some work has been done on other species including its close relative, *Armillaria ostoyae*, which is an aggressive pathogen of conifer trees. *Armillaria ostoyae* has been documented as causing mortality of single or small groups of seedlings or saplings (Morrison 1981, Mallett and Hiratsuka 1985). 'Disease centres' (infection centres or disease foci), or areas of dead and dying infected trees (healthy trees may also be present), as small as a few individual trees to as large as 1 ha or more, have been noted in the literature (e.g. Mallett and Hiratsuka 1985, Hood *et al.* 1991, Kile *et al.* 1991) These disease centres may take several different forms, either patches or rings, and can occur on many different scales (Kile *et al.* 1991 and references therein). For example, Kile *et al.* (1991) stated that *Armillaria* infection in mountain pine stands can occur as rings of dying and dead trees that may be up to 120m in diameter. There are other reports in the literature of a clumped spatial pattern in the infection of *Armillaria* species at various scales (Filip and Roth 1977, William and Marsden 1982, Morrison *et al.* 2000). The objective of this chapter was to test for any clumping in spatial location of trees infected by *Armillaria sinapina* within mixedwood forest stands in north-western Alberta to see if patches of infection could be detected within 40 x 40 m plots. Such information, along with the results in Chapter 2, could lead to a better understanding of the process of infection by this potential pathogen and disease spread in mixedwood stands.

Causes of spatial pattern in Armillaria

One of the most commonly cited causes for a clumped infection pattern of *Armillaria* in forests is the spatial pattern of *Armillaria* infected residual stumps (e.g. Hood *et al.* 1991). For example, in young *Pinus radiata* plantations in New Zealand, tree mortality has been observed to decrease with increasing distance from infected stumps (Mackenzie and Shaw 1977, van der Pas 1981). In Ponderosa pine stands, *Armillaria* infection centres were spatially associated with old stumps (Roth *et al.* 1977, 1980). Hood *et al.* (1991) state that patches of infection in Ponderosa pine stands and Douglas-fir stands probably resulted from the vegetative spread of *A. ostoyae* from colonized old stumps (Hood *et al.* 1991).

However, this explanation has not always been found to be satisfactory. For example, in juvenile lodgepole pine stands, Klein-Gebbink *et al.* (1991) did not find a spatial relationship between dead and/or dying trees and stumps even though the stumps in the study all appeared to be colonized by *A. ostoyae*. In a hemlock/ Douglas-fir forest 30 years post-logging, van der Kamp (1995) showed that *A. ostoyae* infection did not decline with distance from stumps.

Another explanation for a clumped distribution of infected trees is that the distribution of the host trees themselves, if clumped, could lead to a clumped distribution of the fungus. Elements of the spatial pattern of trees such as spacing of individual trees, size and spacing of clumps of trees, and density within clumps of trees can have an important influence on a pathogen, as well as other forest species (Doak *et al*. 1992, Dale 1999). van der Kamp (1995) found that trees, overall, were strongly clumped in hemlock/ Douglas-fir stands and that infected trees occurred in smaller clumps. Therefore, in order to learn more about the spatial ecology of *A. sinapina*, we must understand the spatial distribution of its hosts and the spatial distribution of healthy, declining, and dead host plants.

Objectives and questions

The primary objective of this chapter was to describe the spatial pattern of *Armillaria sinapina* infecting live and dead trees (incluidng snags and stumps) within nine 40 x 40 m plots in the boreal mixedwood forest.

The second objective was to examine the possible causes of the observed spatial pattern of *Armillaria sinapina*. So before determining the spatial pattern of *Armillaria sinapina*, the data collected were used to investigate the spatial pattern of the trees, snags, and stumps within the sampled plots. The spatial point pattern of trees, snags and stumps was investigated by examining the spatial pattern of: (1) all trees, snags, and stumps; (2) broadleaf trees, snags, and stumps; and (3) coniferous trees, snags, and stumps; and then asking are broadleaf trees and coniferous trees aggregated, segregated or distributed independently with respect to one another within plots? Further, the spatial point pattern of the *A. sinapina* infecting those trees, snags, and stumps was investigated by asking: what is the spatial pattern of: (1) infected trees snags, and stumps; and (2) uninfected trees, snags, and stumps, and then asking whether uninfected and infected trees were aggregated, segregated or distributed independently of one another within plots? By exploring these questions I could tell whether *A. sinapina* has a clumped distribution within these plots or not and potentially something about the underlying cause(s).

A third objective was to examine patterns in tree decline and death. I wanted to know whether there is a detectable pattern of tree decline and death and whether this pattern is similar to any pattern in infected trees, snags, and stumps determined by the analyses for the first objective. This objective was approached by examining the spatial pattern of: (1) healthy trees; and (2) unhealthy trees (includes declining and dead trees); and determining whether healthy and unhealthy trees were aggregated, segregated or distributed independently of one another within plots.

Methods

Data collection

The overstory forest types consisted of mature coniferous and broadleaf trees at the EMEND study site, a large research project in North-western Alberta (Township 89-90, Range 03, west of the 6th meridian) (see Chapter One page 11 for a detailed description of the study area). In May 1999, all trees, snags and stumps greater than 5cm in diameter and/or taller than 1 m in height were mapped in nine randomly located 40×40m (0.16-ha) study plots. 50 m tapes were run out from the south-west corner of the plot (see Figure 1.3). Each tree was categorized as broadleaf or coniferous and in mid- June each tree was assigned to one of the following health categories: 1) healthy, 2) declining (i.e. visible outer signs of decline in broadleaf trees were yellowing and loss of leaves from the top part of the crown or half a crown present, visible outer signs of decline in coniferous trees were the loss of many needles, needles turning red, half the crown missing or copious amounts of resin seeping from the base of tree); 3) snag (i.e. rooted dead trees that were greater than 1.3 m but less than 10 m in height but this category also includes rooted dead trees that were greater than 10m tall); and 4) stumps (i.e. rooted dead trees that were less than 1.3 m in height). For some analyses all declining trees, snags and stumps were grouped into one category termed "unhealthy trees". Healthy and unhealthy trees were further classified as either infected or not infected. Infection by *A. sinapina* in healthy and unhealthy trees was determined by tracing a rhizomorph on roots and root collars from the outer bark into the cambium layer where a mycelial fan was present (Figure 1.2).

Data Analysis

Where data are taken as points in space (e.g. a map of trees in a plot), as in this study, there are several different methods of analysis that may be used to describe the spatial pattern of those points (Dale 1999). The chosen method was Ripley's K analysis (see Appendix II for a full description of the method). Ripley's K-function was used to analyse spatial patterns within the nine plots. The following univariate datasets were analysed per plot: 1) all healthy and unhealthy trees (includes declining trees, snags and stumps), 2) healthy trees only; 3) snags and stumps only; 4) healthy trees and unhealthy trees, declining trees, snags and stumps that were infected; 5) all broadleaf trees, snags and stumps; and 6) all coniferous trees, snags and stumps in each of the nine plots.

Ripley's K(*t*) function considers the distribution of the distances between all pairs of points in the same plane, not just their nearest neighbours, to describe the spatial pattern (Ripley 1977, Diggle 1983). The K(*t*) function is defined as the expected number of points within a distance *t* of a randomly chosen point in a plot. If the points are randomly arranged as a Poisson distribution then $K(t) = \pi t^2$. The empirical function or the unbiased estimator of K(*t*) is $\hat{K}(t)$ which incorporates Haase's (1995) edge correction and is defined as

$$\hat{K}(t) = A \sum_{i \neq j}^{n} \sum^{n} w_{ij} I_t(u_{ij}) / n^2$$

where A is the area of the plot; *n* is the number of events in the area A; u_{ij} is the distance between pairs of points; $I_t(u_{ij})$ is the counting function, which is equal to 1 when the distance between u_{ij} is less than or equal to radius *t*, otherwise it is counted as 0; w_{ij} is an edge correction term which assumes that the area outside the plot boundary has a point density and distribution pattern similar to that closest to the adjacent edge within the plot; and the summation is for all pairs of points within a circle of radius *t* (Ripley 1977, Diggle 1983, Upton and Fingleton 1985). The interval of *t* or circle size is user defined and the estimate of K(*t*) is recalculated for each value of *t* chosen.

A much easier way of graphing and interpreting the estimates of the Ripley's K(*t*) function is to perform a square root transformation which linearizes $\hat{K}(t)$. The square root sign and π make $\hat{L}(t)$ a linear function of *t*.

This also stabilizes the variance and $\hat{L}(t)$ has an expected value of 0 under a Poisson distribution which gives:

$$\hat{L}(t) = t - \sqrt{\hat{K}(t)/\pi}$$

Plots of $\hat{L}(t)$ versus t were used to investigate the pattern of tree distributions at intervals of 0.5 m up to 10 m. The observed distribution of $L(t)$ was compared with values obtained from Monte Carlo simulations generated from a model of complete spatial randomness where the positions of all the points were randomised, but the number of points were kept constant and the function was recalculated. 95% confidence intervals were generated from 100 randomisations. Any point at which the observed value of $\hat{L}(t)$ exceeded the confidence envelope was considered significantly different than random. Significant positive values over the upper confidence interval indicate the points are over-dispersed whereas a significant negative value under the lower confidence interval indicates the points are clumped.

The bivariate version of the Ripley's $K(t)$ function (see appendix II for a detailed explanation) is recommended for the analysis of spatial association between two different types of objects (e.g., kinds of plants) (Andersen 1992). The observed value $\hat{L}(t)$ is now distinguished as $\hat{L}_{12}(t)$ because the numbers 1 and 2 represent the bivariate analysis of two kinds of plants. Plots of $\hat{L}_{12}(t)$ versus t were used to investigated the spatial association between the following datasets in intervals of 0.5 m up to 10 m: 1) infected and uninfected trees (including healthy and unhealthy trees); 2) healthy and unhealthy trees; and 3) coniferous and broadleaf trees (including all healthy and unhealthy trees). The null hypothesis of independent distributions between the two kinds of plants was tested by randomisation tests, where the labels of the points (plant species 1 and plant species 2) were randomised, but the positions of the points were kept constant and the function recalculated 100 times. Significant positive values above the upper confidence envelope indicate the two species were segregated whereas significant negative values below the lower confidence envelope indicate the two species were aggregated.

Circle sizes (*t*) (scales) increased from 0.5m in 0.5m steps up to 10m, which means that each dataset was analysed 20 times. The alpha level of 0.05 was not adjusted for this multiple testing, therefore it is likely that some type I errors were made. I examined the possibility of using a Bonferroni correction of the alpha value for the number of circle sizes (*t*) (scales) examined (corrected $\alpha = 0.0025$, 0.5/20) but this proved to be too conservative and probably erred on the side of committing type II errors since all possible patterns indicated by testing at alpha = 0.05 level were no longer detected using the more conservative test.

Results

Univariate (single event) distribution patterns

Trees, snags and stumps – overall pattern

The spatial pattern of trees, snags, and stumps in the nine plots was analysed using the Ripley's K function. Overall, seven plots showed scales of clumping while for two out of the nine plots the spatial distribution was not significantly different from random, at any scale (Plots 5 and 6: Figures 3.14a & 3.15a, Table 3.1). For plot 1, the spatial distribution was significantly clumped at scales of 1.5m, 2.5m, 6.5m, and 7.5m (Figure 3.10a, Table 3.1). For plot 2, the spatial distribution was significantly clumped at scales of 2.0-2.5m, 6.0-6.5m with sharper spikes at 2.0m, 5.0m, and 6.5m (Figure 3.11a, Table 3.1). For plot 3, the spatial distribution was significantly clumped at scales of 8.5-10.0m with a sharper spike 8.5m (Figure 3.12a, Table 3.1). For these three plots the observed line fell only slightly outside of the lower confidence envelope. For plot 4, the spatial distribution was significantly clumped at scales of 0.5-2.5m with a spike at 1.5m, and the observed line fell well outside of the lower confidence envelope (Figure 3.13a, Table 3.1). In addition, there was some weaker indication of clumping at a scale of 4.0-4.5m with a spike at 4.0m. For plot 7, the spatial distribution was significantly clumped at two scales 0.5-3.0m with a peak at 3.0 and 4.0-7.5m with a peak at 6.5m and the observed line fell well outside of the lower confidence envelope (Figure 3.16a, Table 3.1). For plot 8 the spatial distribution was significantly clumped at scales of 4.0-10.0m with spikes at 6.5m and

8.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.17a, Table 3.1). In addition, there was some evidence of clumping at a scale of 2.5-4.0m with a spike at 3.5m. Finally for plot 9, there was reasonably strong evidence of a clumped spatial distribution at scales of 5.0m to 10.0m with spikes at 6.0m and 7.5m (Figure 3.18a).

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Healthy trees

There was no single clear pattern of spatial dispersion for healthy trees but the results were somewhat stronger in terms of suggesting clumped dispersion. One plot showed both clumping and overdispersion at different scales, three plots showed clumping, two plots showed overdispersion, and for the remaining three plots the spatial distribution was not significantly different from random, at any scale (Plots 1, 5, and 6: Figures 3.10b, 3.14b, & 3.15b, Table 3.1). For plot 2, the spatial distribution was significantly clumped at a scale of 2.0-2.5m with a spike at 2.0m (Figure 3.11b, Table 3.1). For plot 3, the spatial distribution was significantly overdispersed at a scale of 2.0m (Figure 3.12b, Table 3.1). For these two plots the observed line fell only slightly outside the confidence envelope. For plot 4, the spatial distribution was significantly clumped at scales of 0.5-5.0m with spikes at 1.5m and 4.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.13b, Table 3.1). For plot 7, the spatial distribution was significantly clumped at scales of 4.0-10.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.16b, Table 3.1). In addition, there was weaker evidence of clumping at a scale of clumping at 2.0-3.0m with a spike at 3.0m. For plot 8, the spatial distribution was significantly overdispersed at scales 1.5-2.0m with a spike at 2.0m but the observed line only slightly fell outside of the upper confidence envelope (Figure 3.17b, Table 3.1). Finally, for plot 9, the spatial distribution was significantly clumped at scales of 5.0-7.5m with spikes at 6.0m and 7.5 m and the observed line fell well outside of the lower confidence envelope (Figure 3.18b, Table 3.1). In addition, there was some evidence of overdispersion at a scale of 1.5m but the observed line only slightly fell outside of the upper confidence envelope.

Unhealthy (includes declining trees, snags and stumps)

For the spatial dispersion of unhealthy trees six plots showed evidence of clumping and three plots showed weak evidence for overdispersion. The spatial pattern of declining trees, snags, and stumps showed that for plot 1, the spatial distribution was significantly clumped at scales of 0.5-3.0m with spikes at 1.5m and 5.0m but the observed line fell only slightly outside of the lower confidence envelope (Figure 3.10c, Table 3.1). For plot 2, the spatial distribution was significantly clumped at scales of 4.0-10.0m with spikes at 5.0m and 6.0m, and the observed line fell well outside of the lower confidence envelope (Figure 3.11c, Table 3.1). In addition, there was some weaker evidence of clumping at a scale of 1.5-3.0m with spikes at 0.5m, 1.5m, and 2.5m. For these next five plots the evidence for spatial patterning was weaker because the observed line fell only slightly outside of the confidence envelope. For plot 3, the spatial distribution was significantly overdispersed at a scale of 1.5m (Figure 3.12c, Table 3.1). For plot 4, the spatial distribution was significantly overdispersed at scales of 8.0-10.0m with a spike at 6.0m

(Figure 3.13c, Table 3.1). For plot 5, the spatial distribution was significantly clumped at a scale of 0.5m,(Figure 3.14c, Table 3.1). For plot 6, the spatial distribution was significantly overdispersed at a scale of 2.0m (Figure 3.15c, Table 3.1). For plot 7, the spatial distribution was significantly clumped at two scales 3.0-4.0m with a spike at 3.0 and 6.0-6.5m with a peak at 6.5m (Figure 3.16c, Table 3.1). For plot 8 the observed line fell well outside of the lower confidence envelope suggesting a clumped spatial distribution at scales of 1.5-10.0m and (Figure 3.17c, Table 3.1). Finally, for plot 9, the spatial distribution was significantly clumped at scales of 4.5-10.0m with a spike at 7.5m, and the observed line fell well outside of the lower confidence envelope (Figure 3.18c, Table 3.1).

Broadleaf trees, snags, and stumps

In contrast to the results for all trees, snags and stumps the evidence for spatial patterning in broadleaf trees, snags and stumps was less clear. One plot showed both clumping and overdispersion at different scales, four plots showed evidence of clumping, one plot showed weak evidence for overdispersion and for three out of the nine plots the spatial distribution was not significantly different from random, at any scale (Plots 1, 3, and 5: Figures 3.10d, 3.12d, & 3.14d, Table 3.1). For plot 2, the spatial distribution was significantly clumped at scales of 0.5-2.0m with a spike at 0.5m (Figure 3.11d, Table 3.1). For plot 4, the spatial distribution was significantly clumped at scales of 1.0-1.5m with a spike at 4.0m (Figure 3.13d, Table 3.1). In addition, there was overdispersion at a scale of 9.5-10.0m. For plot 6, the spatial distribution was significantly overdispersed at scales of

1.0-2.5m (Figure 3.15d, Table 3.1). For plot 7, the spatial distribution was significantly clumped at scales of 1.5-3.0m with spikes at 1.5m and 3.0m (Figure 3.16d, Table 3.1). In all four of these plots the observed line fell only slightly outside of the confidence envelope. For plot 8, the spatial distribution was significantly clumped at two scales 2.0-4.0m with a spike at 3.5 and 4.0-10.0m with a spike at 6.5m, and the observed line fell well outside of the lower confidence envelope (Figure 3.17d, Table 3.1). Finally, for plot 9, the spatial distribution was significantly clumped at scales of 4.5-10.0m with spikes at 6.0m, 7.5m, and 8.5m, but, again, the observed line only slightly fell outside of the upper confidence envelope (Figure 3.18d, Table 3.1).

Coniferous trees, snags and stumps

As for all trees, snags, and stumps the results for coniferous trees, snags, and stumps tended to suggest a clumped dispersion. Overall, seven plots showed scales of clumping and for two out of the nine plots the spatial distribution was not significantly different from random, at any scale (Plots 1 and 6: Figures 3.10e & 3.15e, Table 3.1). For plot 2, the spatial distribution was significantly clumped at scales of 3.5-10.0m with spikes at 4.0m, 6.5m and 8.5m and the observed line fell well outside of the lower confidence envelope (Figure 3.11e, Table 3.1). In addition, there was a weaker trend for clumping at a scale of 2.0-3.5m. For plot 3, the spatial distribution was significantly clumped at scales of 8.5-10.0m with a spike at 8.5m, but the observed line fell only slightly outside of the lower confidence envelope (Figure 3.12e, Table 3.1). For plot 4, the spatial distribution was significantly clumped at scales of 0.5-3.0m with a spike at 2.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.13e, Table 3.1). In addition, there was some evidence for clumping at a scale of 3.0-4.5m with a spike at 4.0m. For plot 5, the spatial distribution was significantly clumped at a scale of 0.5m but the observed line fell only slightly outside of the upper confidence envelope (Figure 3.14e, Table 3.1). For plot 7, the spatial distribution was significantly clumped at scales of 4.0-10.0m with spikes at 4.0m and 6.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.16e, Table 3.1). In addition, there was some evidence of clumping at a scale of 3.0-4.0m with a spike at 3.0m. For plot 8, the spatial distribution was significantly clumped at scales of 5.0m and 6.5m but the observed line fell only slightly outside of the upper confidence envelope (Figure 3.17e, Table 3.1). Finally, for plot 9, the spatial distribution was significantly clumped at scales of 4.5-10.0m with a spike at 6.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.18e, Table 3.1). In addition, the results suggested clumping at scales of 1.5-2.0m and 3.5-4.5m but the observed line fell only slightly outside of the lower confidence envelope.

Uninfected trees, snags, and stumps

The results did not provide much strong evidence for spatial patterning in uninfected trees, snags, and stumps. Three plots showed scales of clumping and for six of the nine plots the spatial distribution was not significantly different from random at any scale (Plots 1-3, 5, 6, 8: Figures 3.10f, 3.11f, 3.12f, 3.14f, 3.15f, & 3.17f, Table 3.1). For plot 4, the spatial distribution was significantly clumped at scales of 0.5-3.0m with a spike at

2.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.13f, Table 3.1). In addition, there was some evidence of clumping at scales of 3.5-4.5m with a spike at 4.0m. For plot 7, the spatial distribution was significantly clumped at scales of 1.0-10.0m with spikes at 3.5m and 7.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.16f, Table 3.1). For plot 9, the spatial distribution was significantly clumped at scales of 1.5-2.0m and 5.0-10.0m with spikes at 6.0m and 9.0m but the observed line fell only slightly outside of the upper confidence envelope (Figure 3.18f, Table 3.1).

Infected trees, snags, and stumps

There was no strong evidence for a single pattern of spatial dispersion for infected trees, snags, and stumps. Four plots showed evidence of clumping, three plots showed overdispersion at some scales and for two of the nine plots the spatial distribution was not significantly different from random at any scale (Plots 5 and 9: Figures 3.14g & 3.18g, Table 3.1). For plot 1, the spatial distribution was significantly clumped at scales of 1.0-3.0m, 4.0-7.0m and 8.0-10.0m but the observed fell only slightly outside of the lower confidence envelope (Figure 3.10g, Table 3.1). For plot 2, the spatial distribution was significantly clumped at scales of 2.0-10.0m with spikes at 3.5m and 6.5m and the observed line fell well outside of the lower confidence envelope (Figure 3.11g, Table 3.1). For plot 3, the spatial distribution was significantly overdispersed at a scale of 1.0m and the observed line fell well outside of the upper confidence envelope (Figure 3.12g, Table 3.1). For plot 4, the spatial distribution was significantly overdispersed at a scale of 0.5m but the observed line fell only slightly outside of the upper confidence envelope (Figure 3.13g, Table 3.1). For plot 6, the spatial distribution was significantly overdispersed at a scale of 2.0m but the observed line fell only slightly outside of the upper confidence envelope (Figure 3.15g, Table 3.1). For plot 7, the spatial distribution was significantly clumped at scales of 1.0-9.0m with spikes at 1.5m, 2.5m and 6.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.16g, Table 3.1). Finally, for plot 8, the spatial distribution was significantly clumped at scales of 2.5-9.5m with spikes at 3.5m, 5.0m, 6.5m and 8.0m and the observed line fell well outside of the lower confidence envelope (Figure 3.17g, Table 3.1).

Bivariate (two events) distribution patterns

Broadleaf and coniferous trees, snags and stumps

Overall, three plots showed scales of segregation between broadleaf vs coniferous trees, snags, and stumps and for six out of the nine plots the two were distributed independently with respect to each other (Plots 1-3, 6, 7, and 9: Figures 3.19a, 3.20a, 3.21a, 3.24a, 3.25a, & 3.27a, Table 3.1,). For plots 4, 5, and 8, the broadleaf and coniferous trees were segregated at scales from 1.5-2.5 m with a spike at 2.0m, 7.0-8.5m with spikes at 0.5m and 8.5m and 3.5-6.0m with a spike at 5.0m, respectively, but the observed line fell only slightly outside of the upper confidence envelope (Figures 3.22a, 3.23a, & 3.26a, Table 3.1).

Healthy and unhealthy trees, snags and stumps

Overall the results suggested that healthy trees vs unhealthy trees, snags, and stumps were distributed independently with respect to one another. For seven out of the nine plots there was no evidence of significant spatial patterning (Plots 2-3, and 5- 9: Figures 3.20b, 3.21b, 3.23b – 3.27b, Table 3.1). For plot 1, the healthy trees and unhealthy trees, snags, and stumps were aggregated at scales from 2.0 m and 6.0-10.0m with spikes at 4.0m, 6.5m, and 7.5m but the observed line fell only slightly outside of the lower confidence envelope (Figure 3.19b, Table 3.1). For plot 4, the healthy trees and unhealthy trees, snags, and stumps were segregated at scales from 1.0-3.0 m with spikes at 2.0m and 3.0m but the observed line fell only slightly outside of the upper confidence envelope (Figure 3.22b, Table 3.1).

Infected and uninfected trees, snags, and stumps

Overall, the results suggested that infected vs uninfected trees, snags, and stumps were mainly distributed independently with respect to each other. For five out of the nine plots there was no evidence of significant spatial patterning between the two (Plots 1-3, 6 and 8: Figures 3.19c, 3.20c, 3.21c, 3.24c, & 3.26c Table 3.1). Two of the other plots showed evidence for segregation, one showed both segregation and aggregation at different scales, and one showed evidence for aggregation. For plot 4, the infected and uninfected trees, snags, and stumps were segregated at scales from 1.5-3.0m with a spike at 2.0m and 7.0-8.0m with a spike at 8.0m but the observed line fell only slightly outside of the upper confidence envelope (Figure 3.22c, Table 3.1). For plot 5, again there was weak evidence for segregation at scales from 1.0-1.5m with a spike at 1.5m (Figure 3.23c, Table 3.1) along with weak evidence for aggregation at scales from 5.5-6.5m (Figure 3.23c, Table 3.1). For plot 7, the infected and uninfected trees, snags, and stumps were segregated at two scales from 1.0-5.0m and 5.5-10.0m and the observed line fell well outside of the upper confidence envelope (Figure 3.25c, Table 3.1). Finally, for plot 9, the infected and uninfected trees, snags, and stumps were aggregated at scales from 4.0-10m and the observed line fell well outside of the lower confidence envelope (Figure 3.26c, Table 3.1). In addition, there was weak evidence for aggregation at scales from 2.0-3.0m (Figure 3.26c, Table 3.1).

Discussion

Spatial point pattern of all trees, snags, and stumps

Trees within a stand are not usually randomly distributed and there are often several scales of spatial pattern present (Moeur 1993, Dale 1999, Mast and Veblen 1999). The distribution of trees within a stand may be affected by a wide variety of factors including regeneration processes, facilitation, density dependent self-thining, and site conditions such as edaphic variation, microclimatic variation, disturbance history (e.g. fire, windthrow, etc), and biotic factors (e.g. herbivory, insects, and pathogens) (Harper 1977, Augspurger and Kelley 1984, Peet and Christensen 1987, Moeur 1993, Crawley 1997, Dale 1999). Because the distribution of a pathogen heavily depends on the distribution of its host, the spatial point patterns of *Armillaria* infection we detected must be interpreted in the light of the spatial pattern of the hosts themselves. The spatial point pattern analysis showed that for most plots, the overall pattern for trees, snags, and stumps tended to be clumped at relatively small scales (less than 10 m), with a few exceptions.

This is not surprising because many analyses of forest trees show clumping at similar scales (e.g. Peterson and Squiers 1995). Over time this clumping may progress toward overdispersion through self-thinning (Sterner *et al.* 1986, Kenkel 1988) and there was some evidence that this might be occurring in a couple of the plots (plots 1 and 8). For these plots, all trees overall were clumped, but the unhealthy and the dead trees were clumped and the healthy trees were either random or overdispersed. For the remaining plots, results were variable and there was no evidence for the process of self thinning being important in terms of an affect on spatial pattern.

In theory, it is expected that plant competition, which includes any interaction that reduces plant fitness of both competitors, should produce overdispersed patterns of mature individual plants (Greig-Smith 1957). However, there are relatively few accounts of overdispersed tree spatial patterns in the literature (Kenkel 1988) but studies have shown that initially clumped spatial patterns have shifted over time to a random pattern and this has been presented as evidence of movement towards overdispersion (e.g. Peet and Christensen 1987, Kenkel 1988, He *et al.* 1997). For pine, spruce, and aspen intraspecific competition, or self-thinning, is thought to be most intense in the initial plant stages (seedlings and saplings) and in post-disturbance situations (Pollard 1971, Kenkel 1988, Newton and Joliffe 1998).

Spatial point pattern of Armillaria sinapina *infecting trees, snags, and stumps*

I expected to be able to detect significant clumps of infected trees, snags and stumps in all of my plots. However, this expectation was based upon the many years of documentation of the clumped pattern of *Armillaria* root disease not on known patterns of *Armillaria sinapina*. *Armillaria* root disease is known to cause 'disease centres', areas of dead and dying trees due to *Armillaria* root disease which range from a group of a few dead trees to tens of hectares (Wargo and Shaw 1985). According to Pielou (1963) infected trees and uninfected trees would be expected to be segreated from one another and infected trees should appear in clumps. Results from a 2.88 acre plantation of 17-year old Douglas-fir trees showed that diseased trees and healthy trees were indeed segregated from one another (Pielou 1963). In the current study, univariate point pattern analysis showed that the patterns of infected trees, snags, and stumps were highly variable and that four out of the nine plots showed clumping of infected trees. Bivariate point pattern analysis of infected versus uninfected trees were also highly variable and three plots showed significant segregation of infected and uninfected trees. These results suggest that there is variability in the distribution of the fungus infecting trees, snags, and stumps at this scale. Since most of the overall tree, snag and stump patterns were clumped, I would have expected that if the fungus was infecting trees in these clumps that groups of trees would also be infected in those clumps. This pattern was not always supported and in fact at these small scales for these nine plots more than half of the plots did not show this pattern. This suggests that there is a random process of infection by *Armillaria sinapina* in these plots.

Spatial point pattern of broadleaf trees, snags, and stumps

Chapter Two showed that *Armillaria sinapina* was more likely to be found on broadleaf host trees than coniferous. Therefore, it makes more sense to interpret the spatial point pattern in the light of the distribution of broadleaf trees rather than all trees. For the broadleaf dominated plots (plots 7, 8, 9), broadleaf trees showed evidence of clumping. For the mixedwood to coniferous dominated plots, the spatial pattern of broadleaf tree, snag, and stump pattern was more varied.

Aspen is an extremely widespread, clonal plant, whose ramets are interconnected by an extensive underground root system. Aspen forms clones that range in size from a few stems up to several hundred stems over several hectares (Johnson *et al.* 1995). Because of this clonal habit, it has been predicted, and shown empirically, that aspen does not exhibit the typical pattern of self-thinning that is observed in stands of other species (see Peterson and Squiers 1995). It vigorously suckers following a stand-replacing disturbance, such as fire or logging, which results in rapid vegetative recruitment and a cohort of ramets that are very close in age (Peterson and Squiers 1995). After the initial surge of sucker establishment, temperature and light are reduced because of shading effects and therefore suckering decreases over time (Peterson and Peterson 1992). This means that the clumping observed in the broadleaf trees in many of the plots may be due to heterogeneity in position of the active clonal roots from which the ramets suckered, or heterogeneity in the disturbance event and its impact on suckering.

Coniferous trees are very different ecologically from broadleaf trees. Conifers are unitary plants meaning that each stem within a stand is a different individual (Harper

1977). For white spruce, the main species of coniferous trees in my plots, the primary mode of reproduction is via seeds (Harper 1977). It is well known that the majority of seeds produced will fall near the parent plant and can lead to clumped distributions of seedlings (Harper 1977). Also, heterogeneity in favourable microsites for establishment is often quite patchy. Therefore, initially, young conifer trees are expected to have a clumped spatial distribution (Peet and Christensen 1987). My results provide some evidence that conifers in these mature stands showed some level of clumped dispersion and this may have contributed to the overall pattern of clumping in all trees, snags, and stumps in conifer-dominated plots.

We can attempt to interpret the spatial patterning of infected trees, snags, and stumps in light of these spatial patterns of coniferous and broadleaf trees in the plots of varying coniferous – broadleaf composition. In conifer-dominated plots (e.g. Plots 1, 2, 3, 4) the spatial pattern of broadleaf trees did not closely match the pattern of infected trees, snags, and stumps. For the broadleaf dominated plots, however, the scales of clumping in infected trees, snags, and stumps tended to overlap with the scales at which there was clumping in broadleaf trees, snags, and stumps. This is likely because *Armillaria sinapina* prefers broadleaf hosts and thus the clumped distribution of infected trees simply reflects the clumped distribution of the broadleaf hosts, with that pattern being most evident in the broadleaf-dominated plots.

One conclusion that may be drawn from these patterns is that the scale of sampling (40 x 40 m plots) was too small to see the spatial pattern of disease centres and that any patches and gaps in the distribution of *Armillaria sinapina* infecting trees, snags, and stumps in these forest stands occur at a larger scale. An alternative conclusion may be simply that *A. sinapina* is distributed ubiquitously throughout these stands because they are rich in prefered substrate types such as broadleaf trees and broadleaf downed woody material and the rhizomorphs can move easily through the organic layer to reach any potential host substrate (Chapter Two).

Tree health assessments for Armillaria sinapina

It is difficult to use the spatial pattern data to come to clear conclusions relating to the relationship between tree health assessments and infection by *Armillaria sinapina*. For most plots unhealthy trees were clumped but for three of the plots they were over dispersed. Also, for most plots, healthy and unhealthy trees were distributed independently of each other. As shown by the results in Chapter Two there was no relationship between unhealthy or dead trees and the availability of *A. sinapina* rhizomorphs or the presence of *A. sinapina* infection. This means that, at these scales for these nine plots, we cannot say whether *A. sinapina* is a significant cause of death and disease in these forest trees. There are two possible explanations for this.

First, it is thought that aspen stems that are disadvantaged in terms of resource acquisition can survive as part of a clone by gaining resources from other ramets in the surrounding clone. This leads to more mature clumps of stems in favourable patches within stands (Peterson and Squiers 1995). This may be why there is little relationship between the dead and unhealthy trees, infection by *A. sinapina*, and *A. sinapina* availability in the organic layer: individual ramets may remain healthy longer even though they are infected because they can rely on resources obtained by other ramets in the same clone.

A second, and probably more likely, explanation is that *A. sinapina* is a ubiquitous long-lived pathogen of low virulence in this system. This fungus may remain in a tree stem until the plant is weakened or killed by another factor or parasite (e.g. windthrow or forest tent caterpillar), at which point the fungus maybe become pathogenic or simply make use of the dead tree resource as a saprophyte.

Summary and conclusions

The purpose of this chapter was to describe the spatial pattern of infection by *A. sinapina* in mixed-wood forests and to find possible explanations for these patterns. The pattern of infection on trees, snags, and stumps was clumped for some of the plots. However, for over half of the plots there was a random pattern of infection on trees, snags and stumps which suggests that *Armillaria sinapina* randomly infects trees, snags, and stumps in these plots at these small scales. For broadleaf dominated plots, the pattern of infection was clumped at scales similar to those at which there was clumping of broadleaf trees and this was, no doubt, a reflection of the distribution of the host.

Table 3.1: Summary table of results from univariate and bivariate spatial point pattern analyses in plots of mixedwood forest. Plots are numbered by increasing dominance by broadleaf trees (see Table 2.1). tss indicates all trees, snags and stumps, Unhealthy dss indicates declining trees plus snags and stumps, ● indicates a clumped pattern and # indicates overdispersion for a univariate pattern; + represents aggregation and - represents segregation for a bivariate pattern; ns indicates no significant spatial pattern was detected (both univariate and bivariate analysis). See Figures 3.10 to 3.21 for complete results.

Plot	Overall tss	Healthy	Unhealthy dss	Hardwood tss	Coniferous tss	Uninfected tss	Infected tss	Hardwood/ Coniferous	Healthy/ Unhealthy	Infected/ Uninfected
1	●	ns	●	ns	ns	ns	●	ns	+	ns
2	●	●	●	●	●	ns	●	ns	ns	ns
3	●	#	#	ns	●	ns	#	ns	ns	ns
4	●	●	#	●	●	●	#	-	-	-
5	ns	ns	●	ns	●	ns	ns	-	ns	- / +
6	ns	ns	#	#	ns	ns	#	ns	ns	ns
7	●	●	●	●	●	●	●	ns	ns	-
8	●	#	●	●	●	ns	●	-	ns	ns
9	●	●	●	●	●	●	ns	ns	ns	+

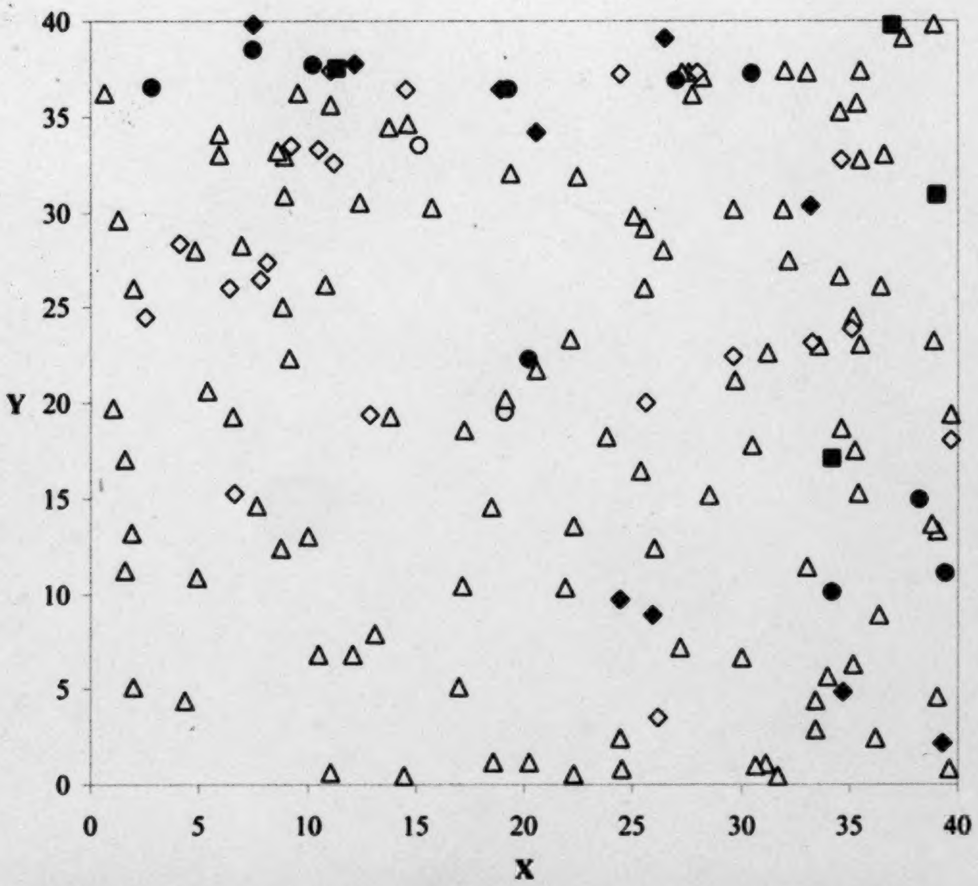

Figure 3.1: Map of plot 1 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (o), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

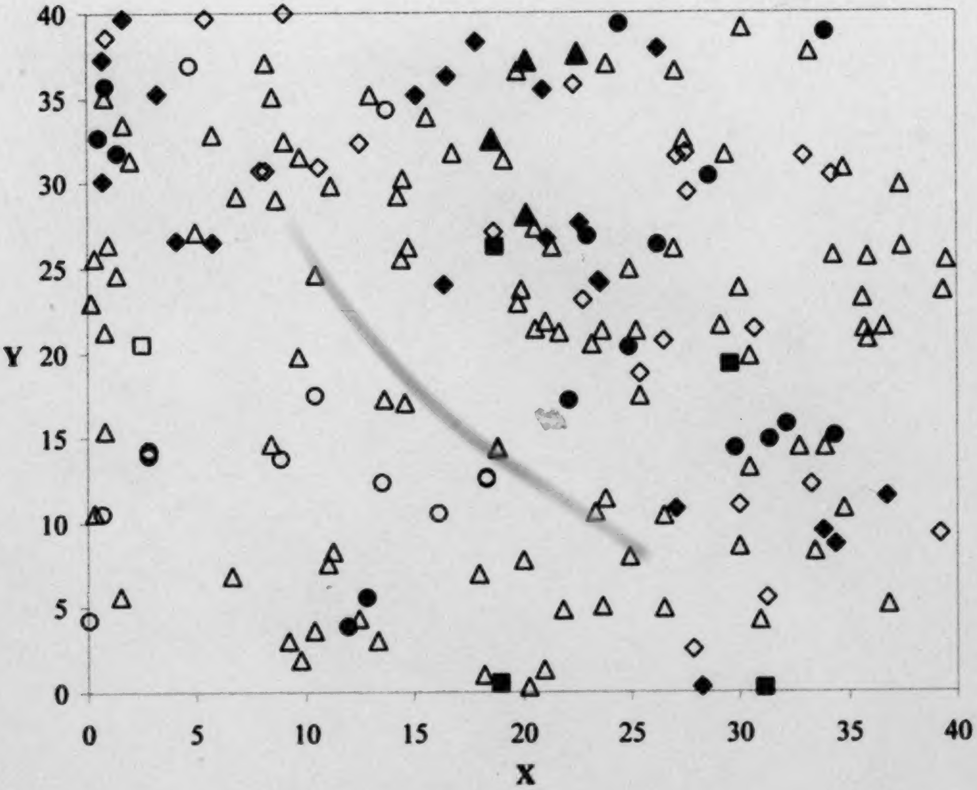

Figure 3.2: Map of plot 2 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (o), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

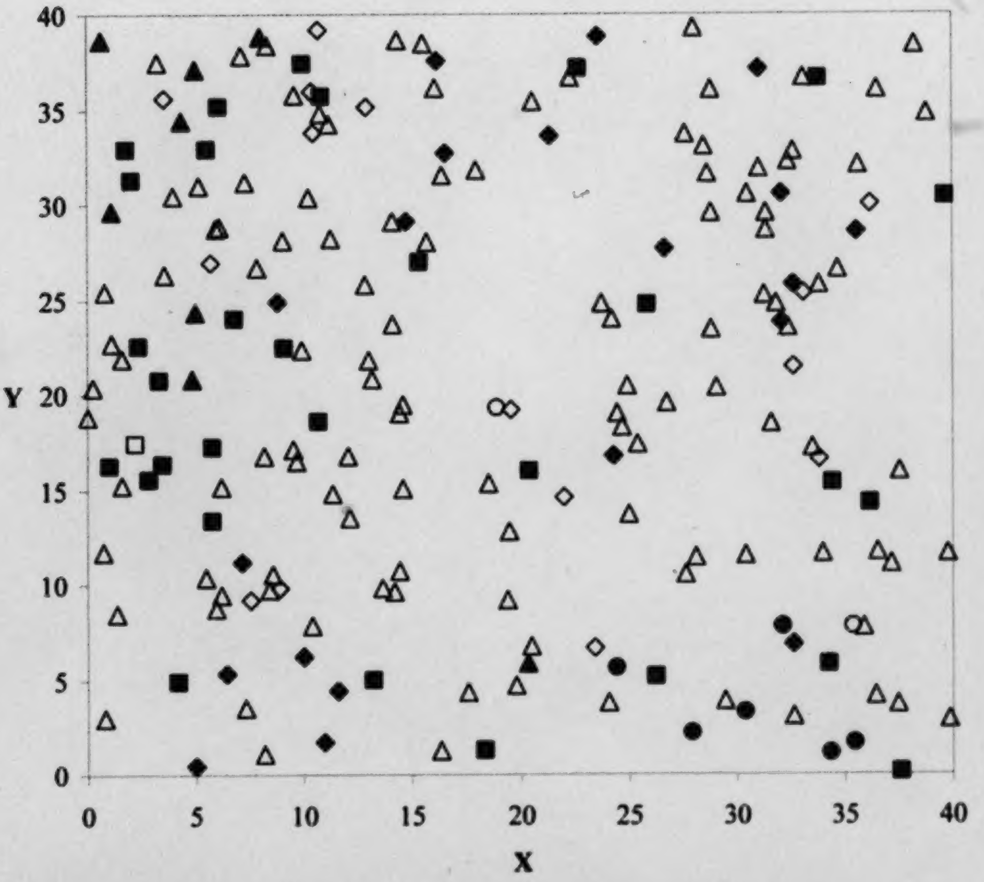

Figure 3.3: Map of plot 3 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (o), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

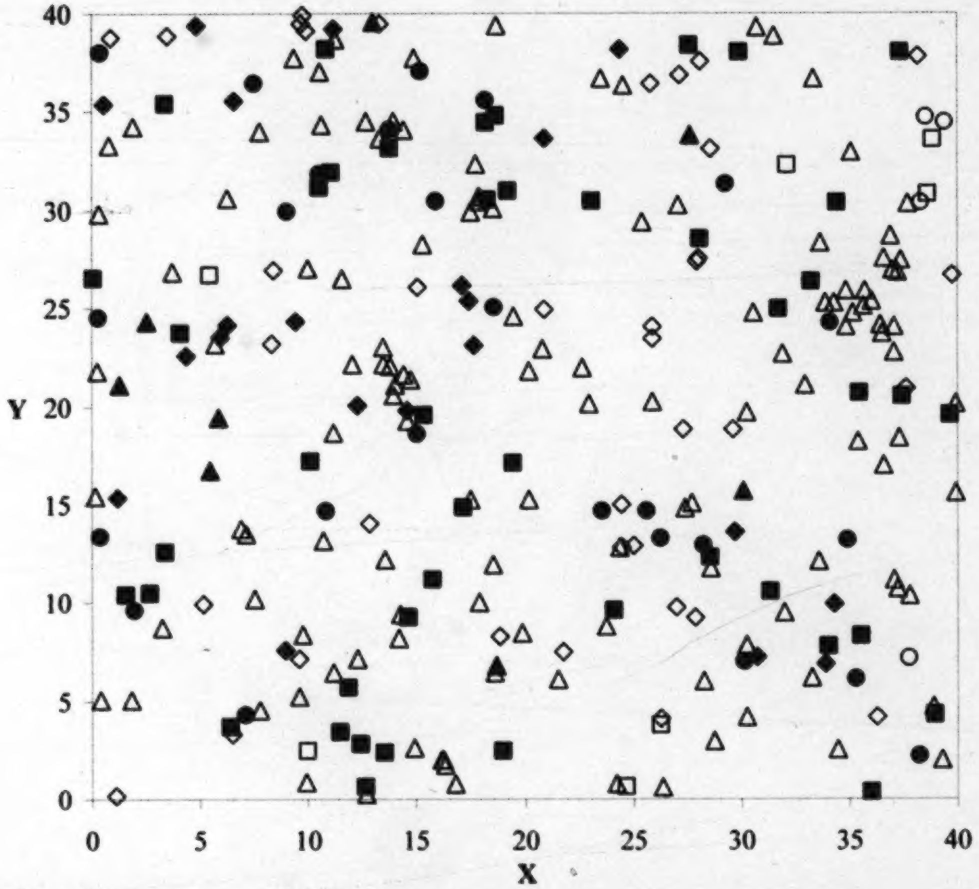

Figure 3.4: Map of plot 4 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (o), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

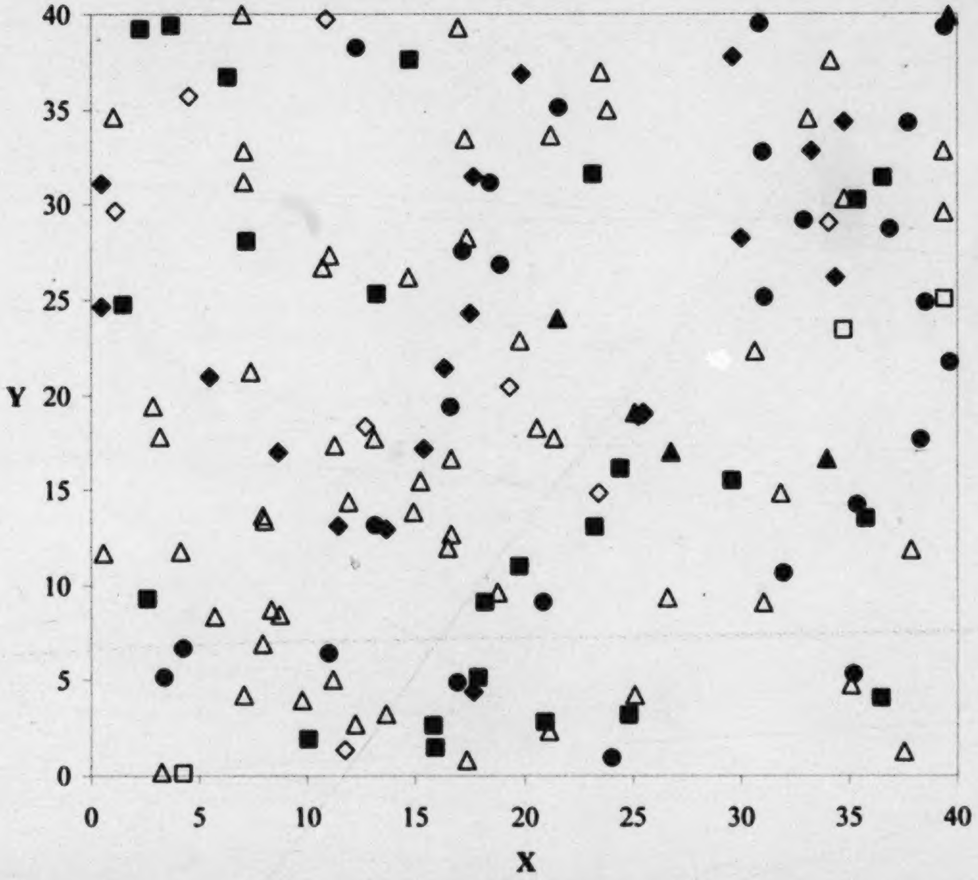

Figure 3.5: Map of plot 5 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (o), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

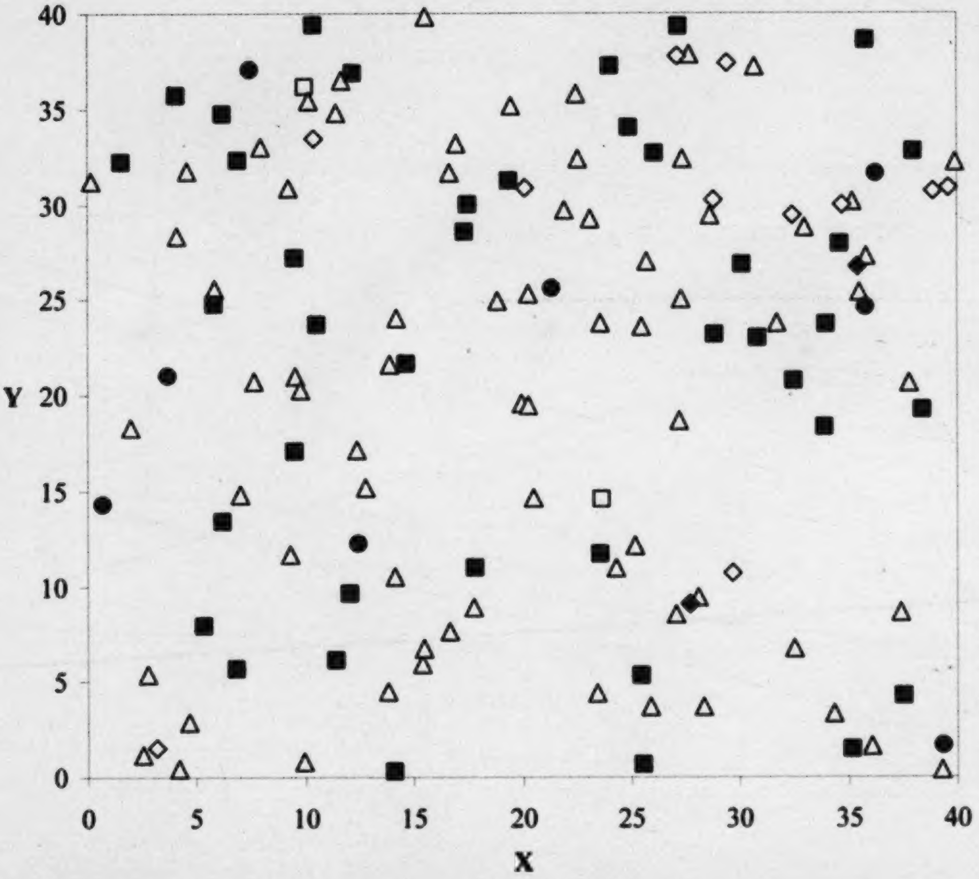

Figure 3.6: Map of plot 6 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (o), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

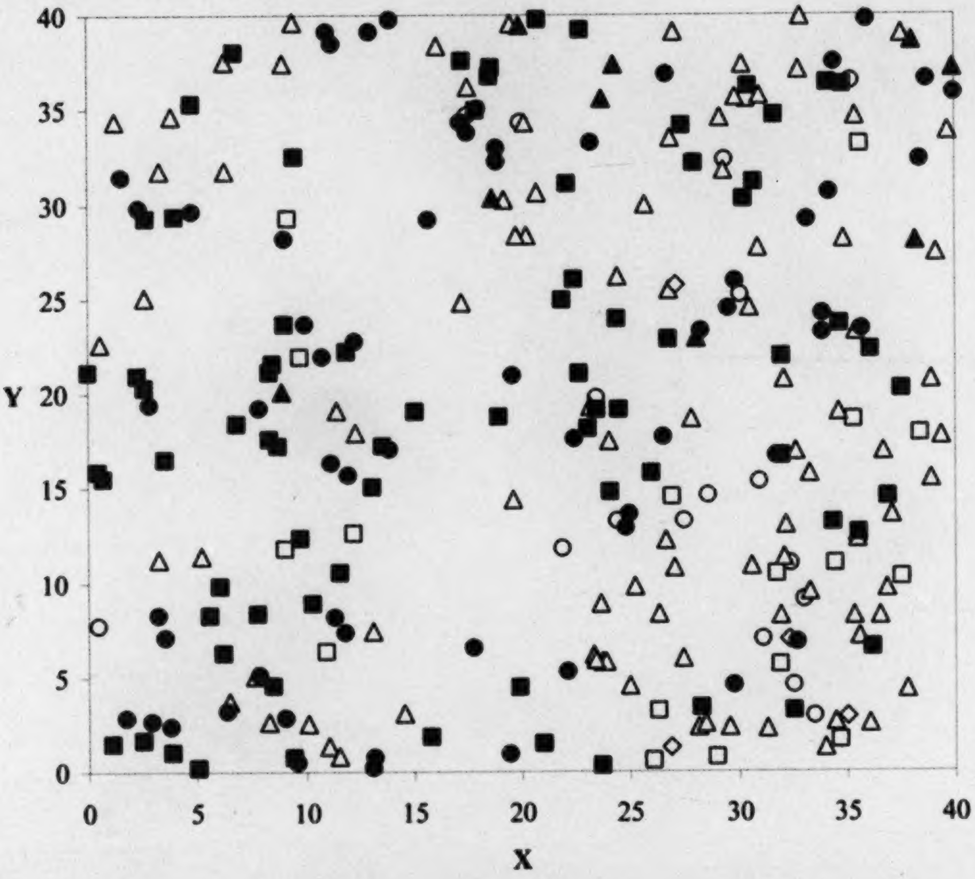

Figure 3.7: Map of plot 7 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (o), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

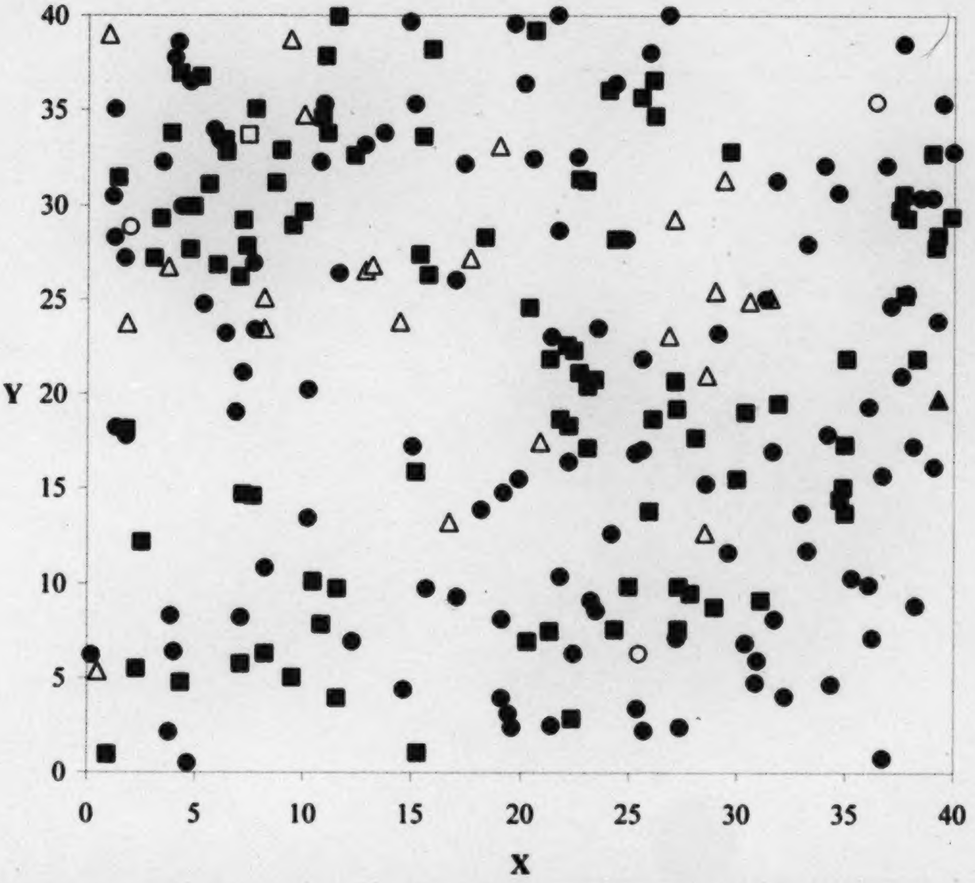

Figure 3.8: Map of plot 8 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (o), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

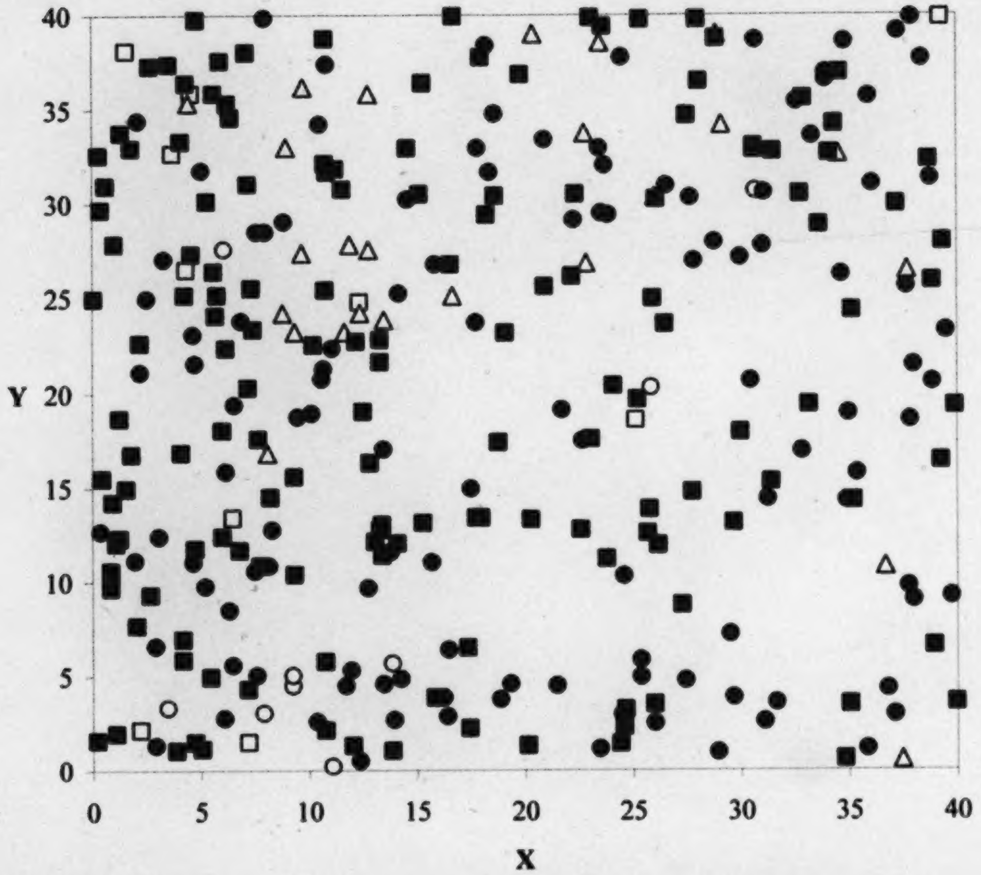

Figure 3.9: Map of plot 9 showing the positions of all trees, snags, and stumps. The blackened symbols represent *Armillaria sinapina* infection; the open symbols represent the absence of *A. sinapina* infection. The symbols also indicate if the substrate was broadleaf or coniferous and if it was healthy or dead as follows: healthy broadleaf trees (●), infected dead broadleaf trees, snags or stumps (tss) (■), infected healthy coniferous trees (▲), infected dead coniferous tss (◆), uninfected broadleaf trees (○), uninfected dead broadleaf tss (□), uninfected healthy coniferous trees (Δ), and uninfected dead coniferous tss (◊).

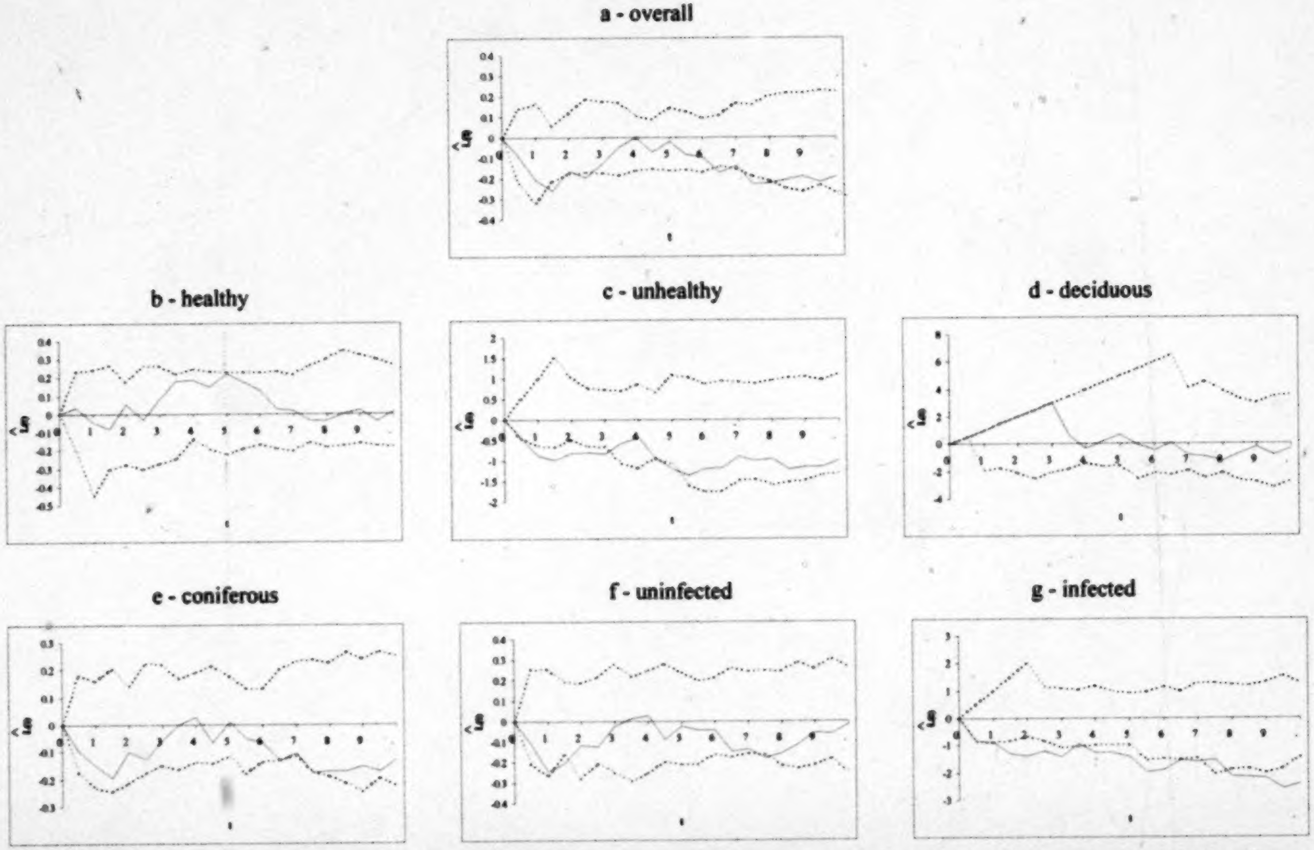

Figure 3.10: Results of Ripley's K univariate analysis for plot 1. Alpha = 0.05. The solid single line represents an estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps (N=153); (b) univariate pattern of healthy trees (N=119); (c) univariate pattern of declining trees, plus snags and stumps (N=34); (d) univariate pattern of broadleaf trees, snags, and stumps (N=11); (e) univariate pattern of coniferous trees, snags, and stumps (N=142); (f) univariate pattern of uninfected trees, snags and stumps (N= 129); (g) univariate pattern of infected trees, snags, and stumps (N=24).

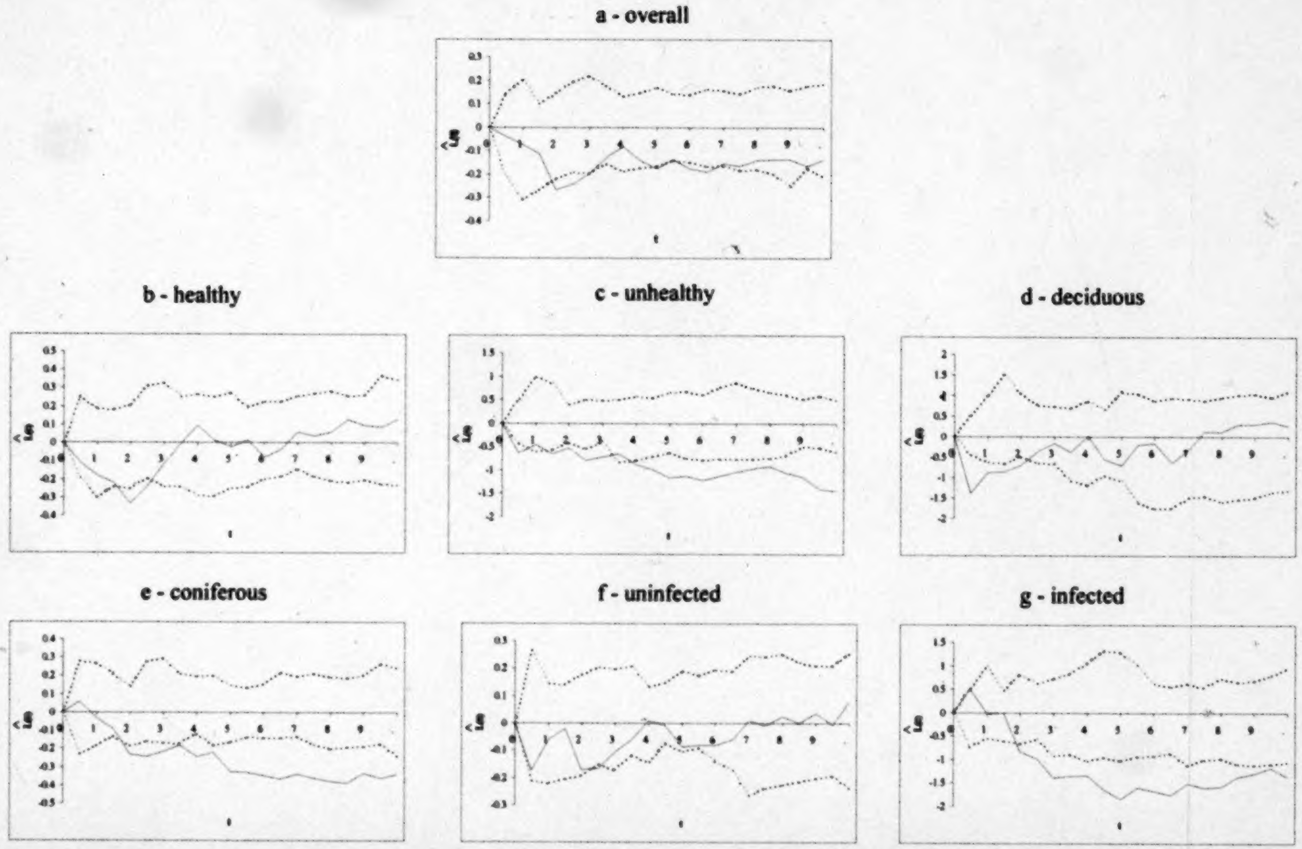

Figure 3.11: Results of Ripley's K univariate graphs for plot 2. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps N=179, (b) univariate pattern of healthy trees N=130, (c) univariate pattern of declining trees, snags and stumps N=49 (d) univariate pattern of broadleaf trees, snags, and stumps N=34 (e) univariate pattern of coniferous trees, snags, and stumps N=145, (f) univariate pattern of uninfected trees, snags and stumps N=55 (g) univariate pattern of infected trees, snags, and stumps N=48.

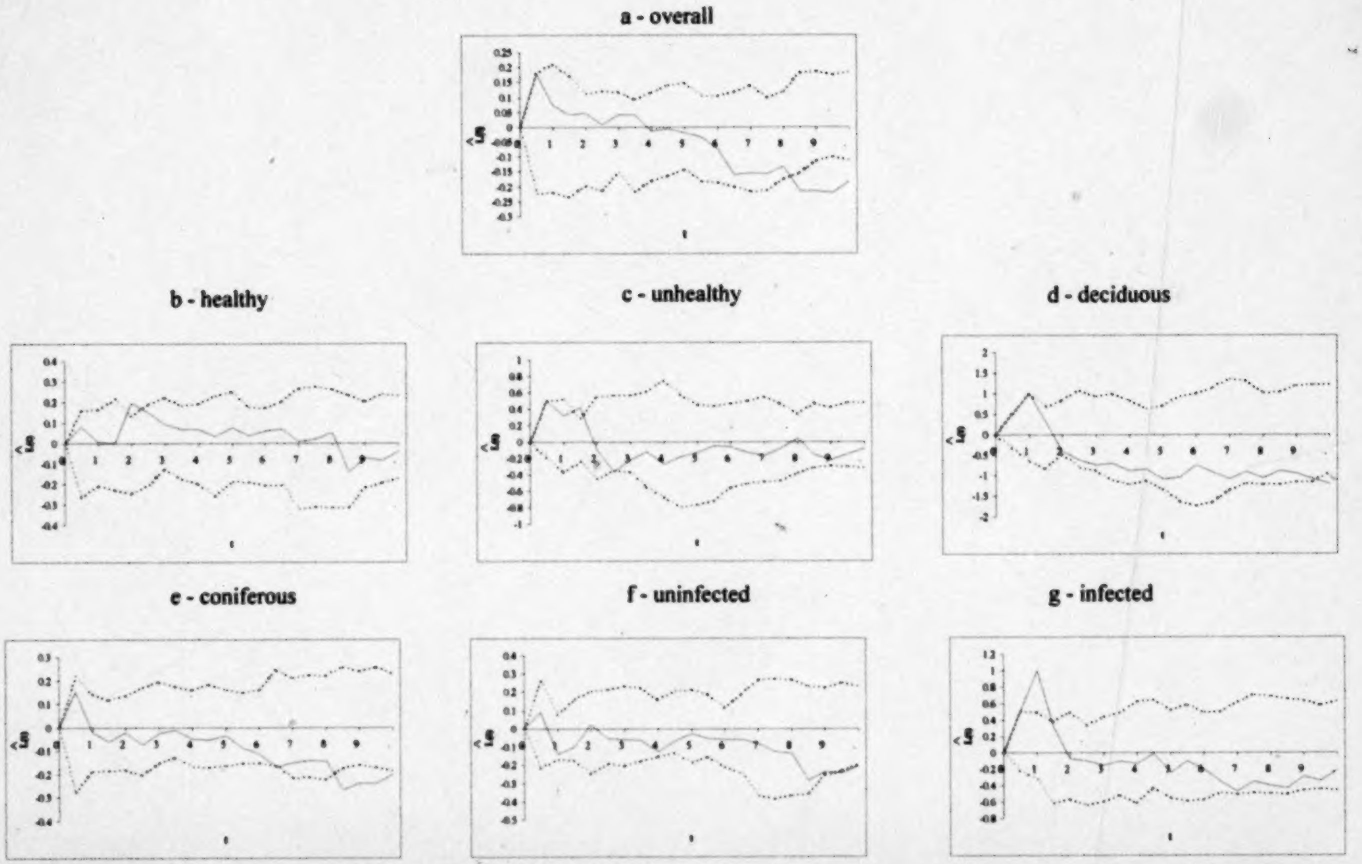

Figure 3.12: Results of Ripley's K univariate graphs for plot 3. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps N=198, (b) univariate pattern of healthy trees N=132, (c) univariate pattern of declining trees, snags and stumps N=66, (d) univariate pattern of broadleaf trees, snags, and stumps N=40, (e) univariate pattern of coniferous trees, snags, and stumps N=158, (f) univariate pattern of uninfected trees, snags, and stumps N=134, (g) univariate pattern of infected trees, snags, and stumps N=64.

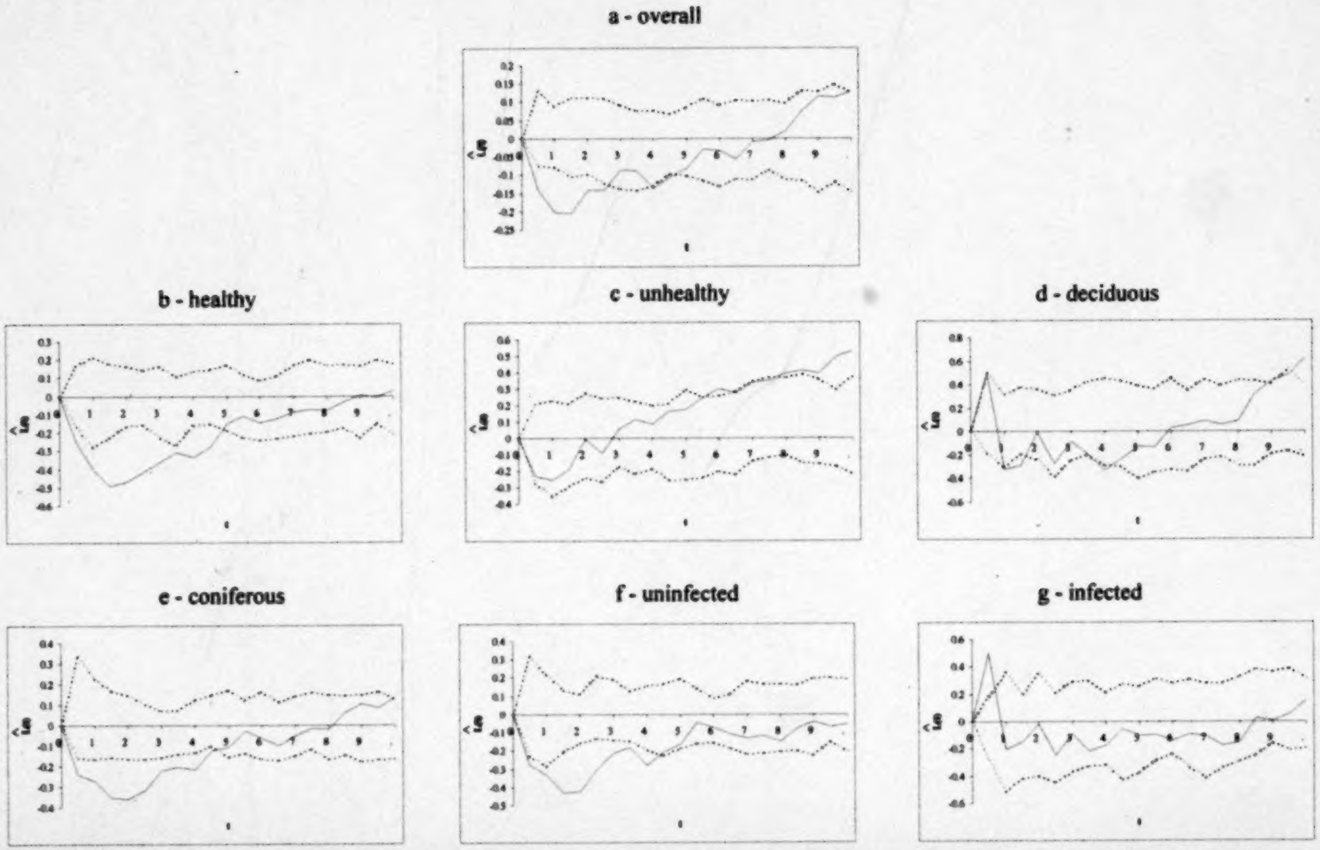

Figure 3.13: Results of Ripley's K univariate graphs for plot 4. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps N=275, (b) univariate pattern of healthy trees N=165, (c) univariate pattern of declining trees, snags, and stumps N=110, (d) univariate pattern of broadleaf trees, snags, and stumps N=81, (e) univariate pattern of coniferous trees, snags, and stumps N=194, (f) univariate pattern of uninfected trees, snags, and stumps N=176, (g) univariate pattern of infected trees, snags, and stumps N=99.

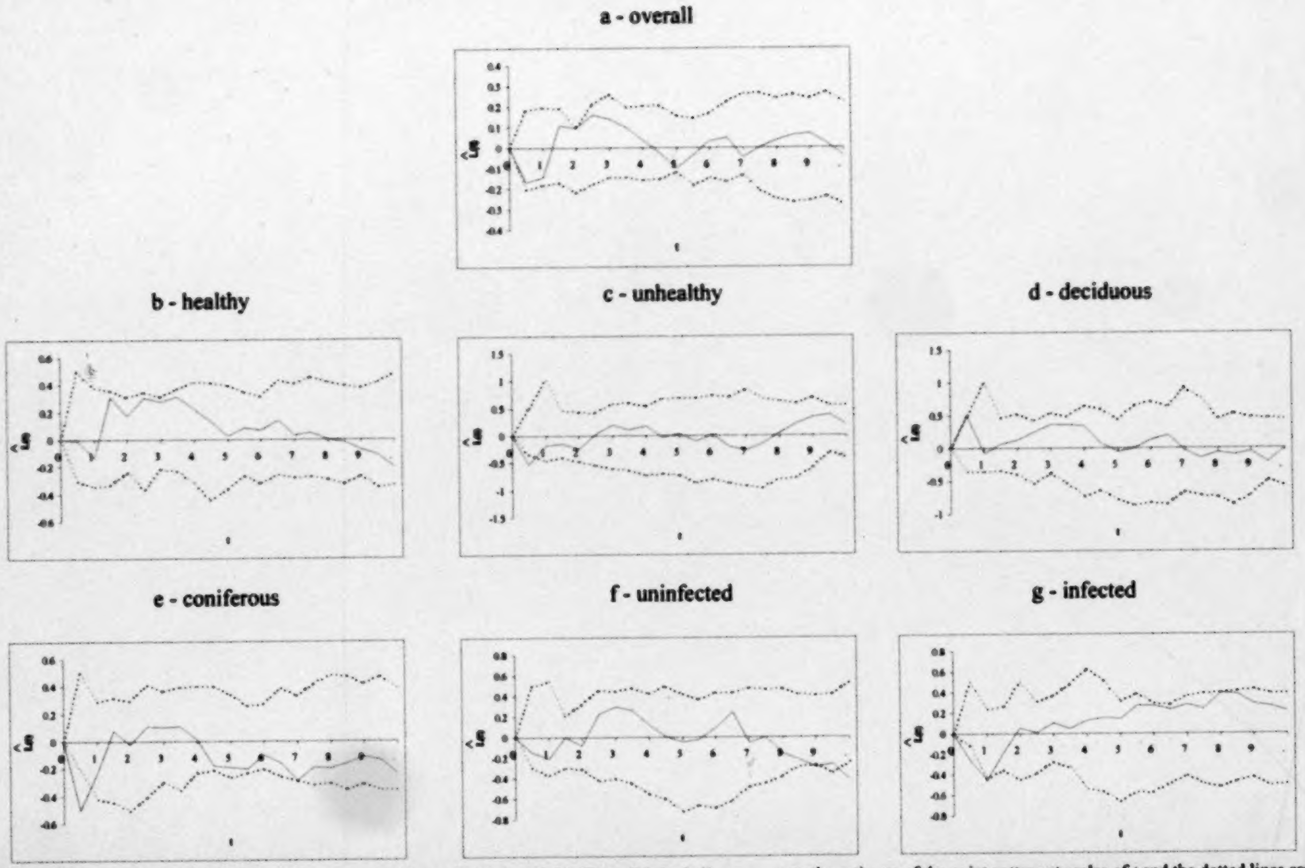

Figure 3.14: Results of Ripley's K univariate graphs for plot 5. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps N=143, (b) univariate pattern of healthy trees N=89, (c) univariate pattern of declining trees, snags and stumps N=54, (d) univariate pattern of broadleaf trees, snags, and stumps N=53, (e) univariate pattern of coniferous trees, snags, and stumps N=90, (f) univariate pattern of uninfected trees, snags, and stumps N=70, (g) univariate pattern of infected trees, snags, and stumps N=73.

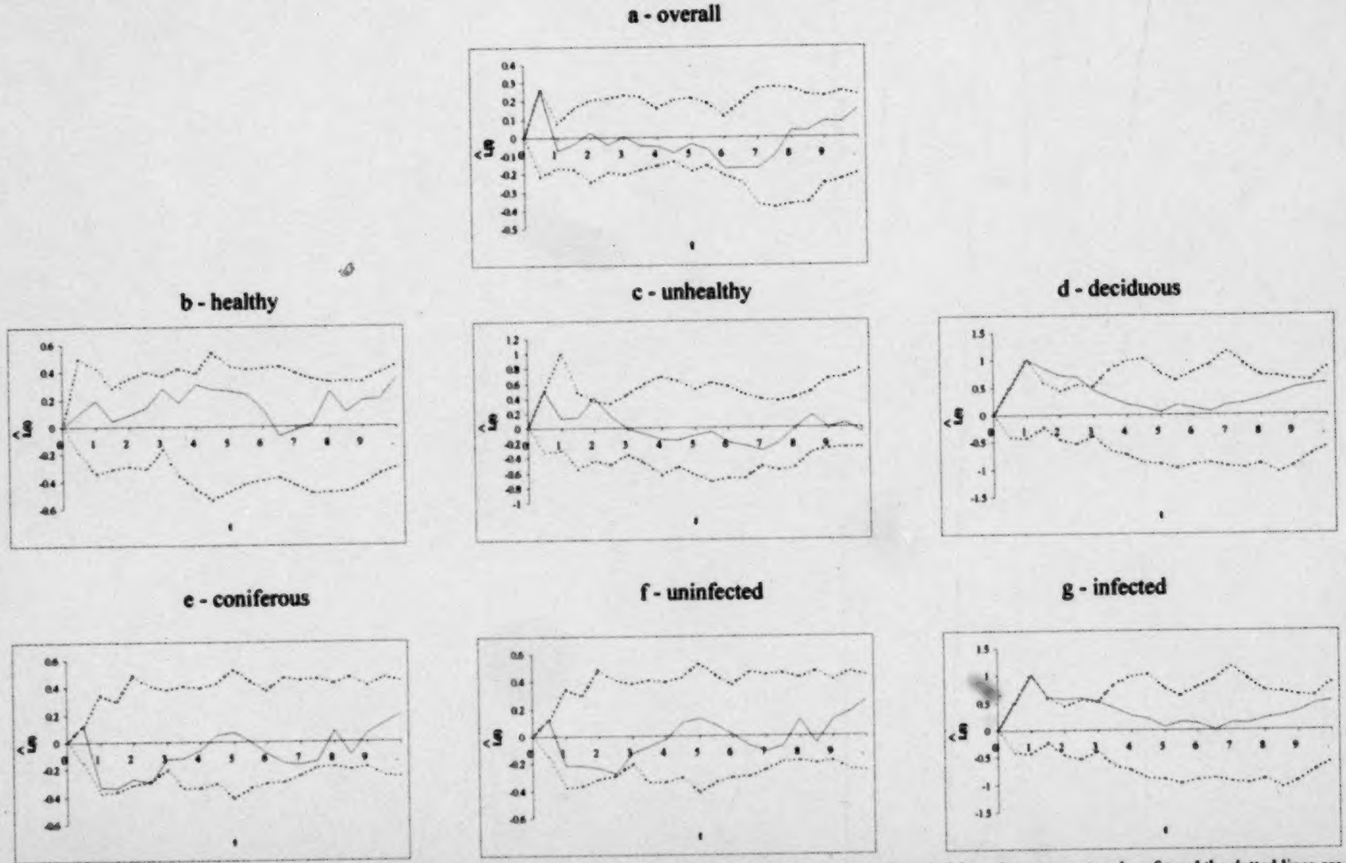

Figure 3.15: Results of Ripley's K univariate graphs for plot 6. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps N=134, (b) univariate pattern of healthy trees N=79, (c) univariate pattern of declining trees, snags, and stumps N=55, (d) univariate pattern of broadleaf trees, snags, and stumps N=51, (e) univariate pattern of coniferous trees, snags, and stumps N=84, (f) univariate pattern of uninfected trees, snags and stumps N=84, (g) univariate pattern of infected trees, snags, and stumps N=50.

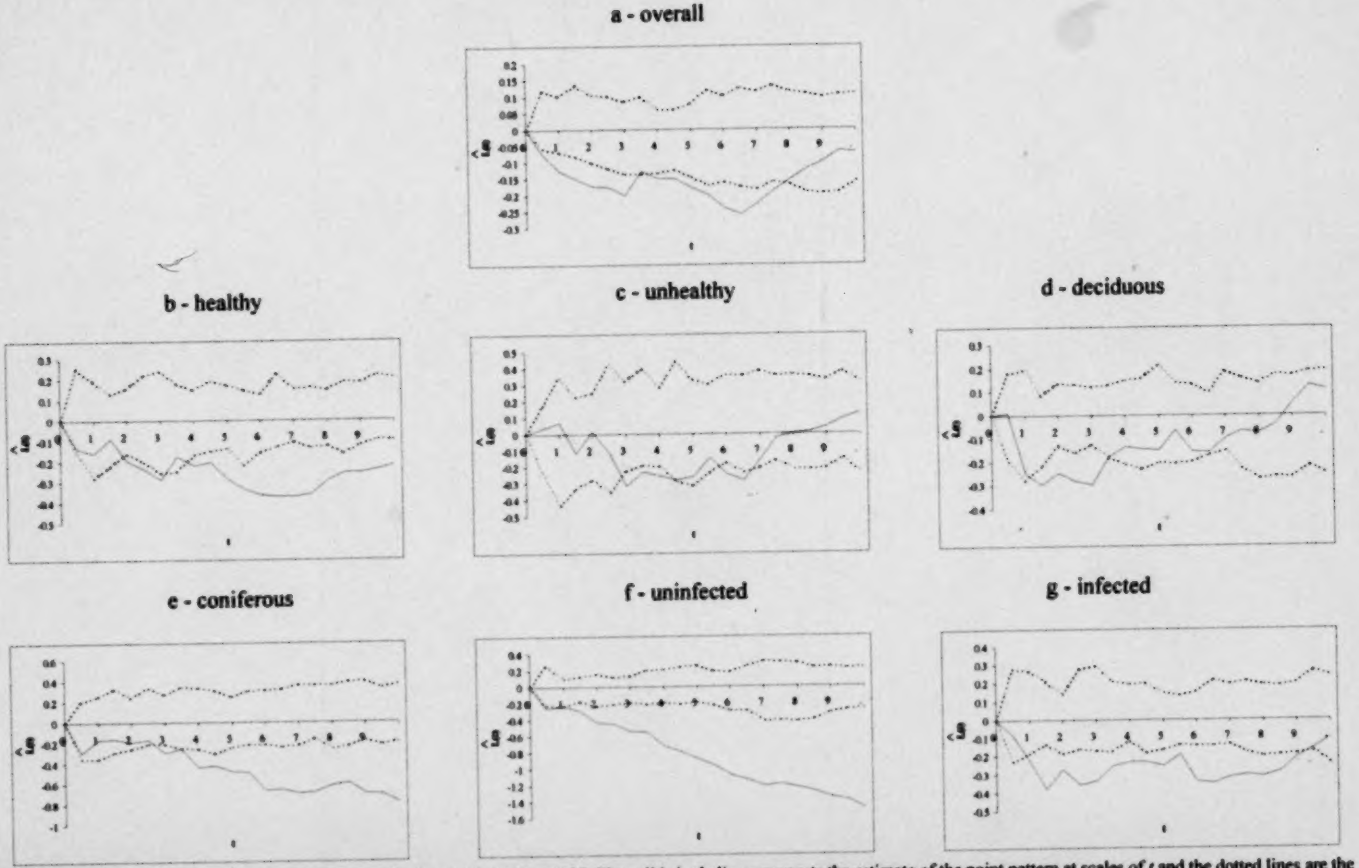

Figure 3.16: Results of Ripley's K univariate graphs for plot 7. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps N=278, (b) univariate pattern of healthy trees N=181, (c) univariate pattern of declining trees, snags, and stumps N=97, (d) univariate pattern of broadleaf trees, snags, and stumps N=172, (e) univariate pattern of coniferous trees, snags, and stumps N= 106, (f) univariate pattern of uninfected trees, snags, and stumps N=133, (g) univariate pattern of infected trees, snags, and stumps N=145.

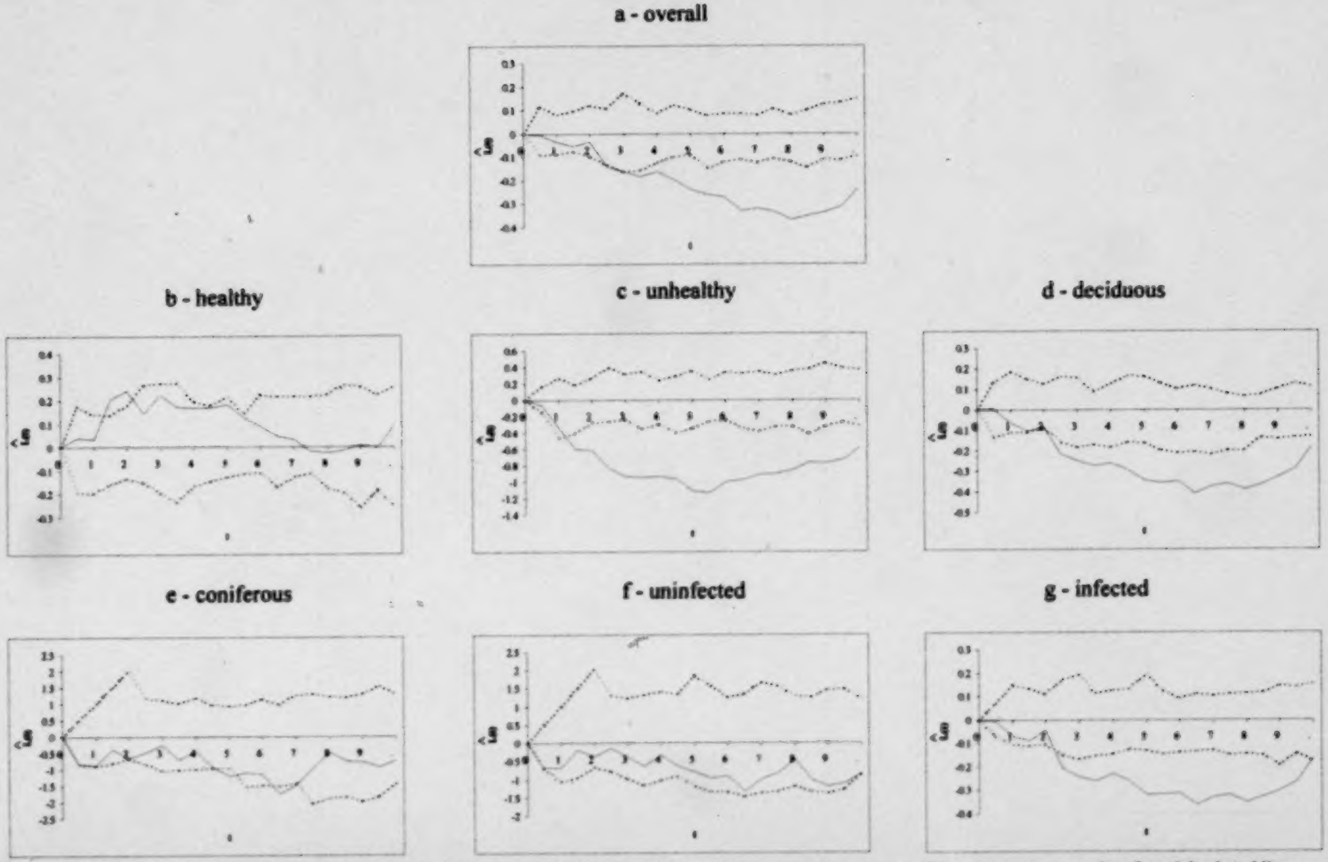

Figure 3.17: Results of Ripley's K univariate graphs for plot 8. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps N=237, (b) univariate pattern of healthy trees N=139, (c) univariate pattern of declining trees, snags, and stumps N=98, (d) univariate pattern of broadleaf trees, snags, and stumps N=213, (e) univariate pattern of coniferous trees, snags, and stumps N=24, (f) univariate pattern of uninfected trees, snags, and stumps N=27, (g) univariate pattern of infected trees, snags, and stumps N=210.

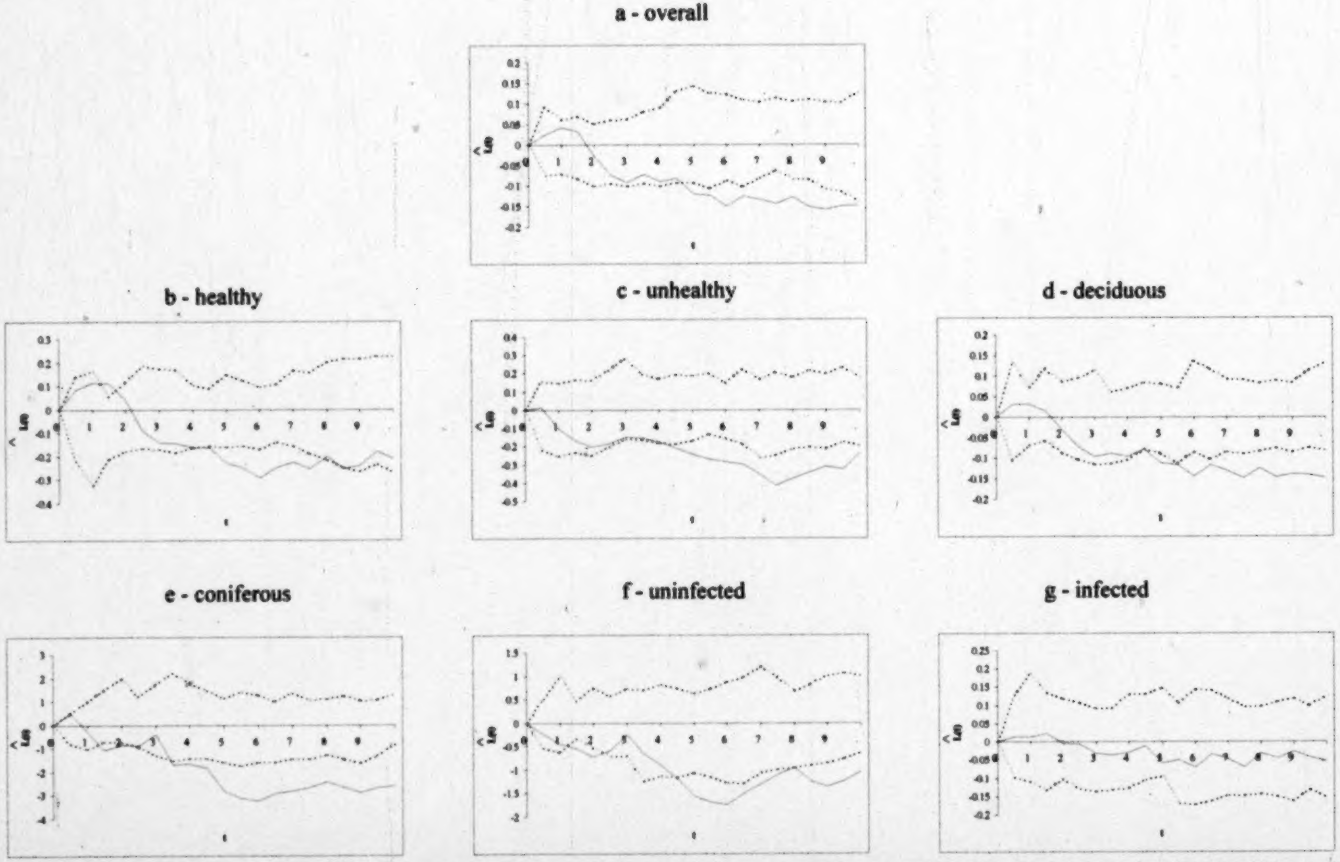

Figure 3.18: Results of Ripley's K univariate graphs for plot 9. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of clumping alternatively if the solid line is above the upper confidence interval it indicates a pattern of overdispersion. (a) univariate pattern of all trees, snags, and stumps N=314, (b) univariate pattern of healthy trees N=153, (c) univariate pattern of declining trees, snags and stumps N=161, (d) univariate pattern of broadleaf trees, snags, and stumps N=279, (e) univariate pattern of coniferous trees, snags, and stumps N=25, (f) univariate pattern of uninfected trees, snags, and stumps N=44, (g) univariate pattern of infected trees, snags, and stumps N=270.

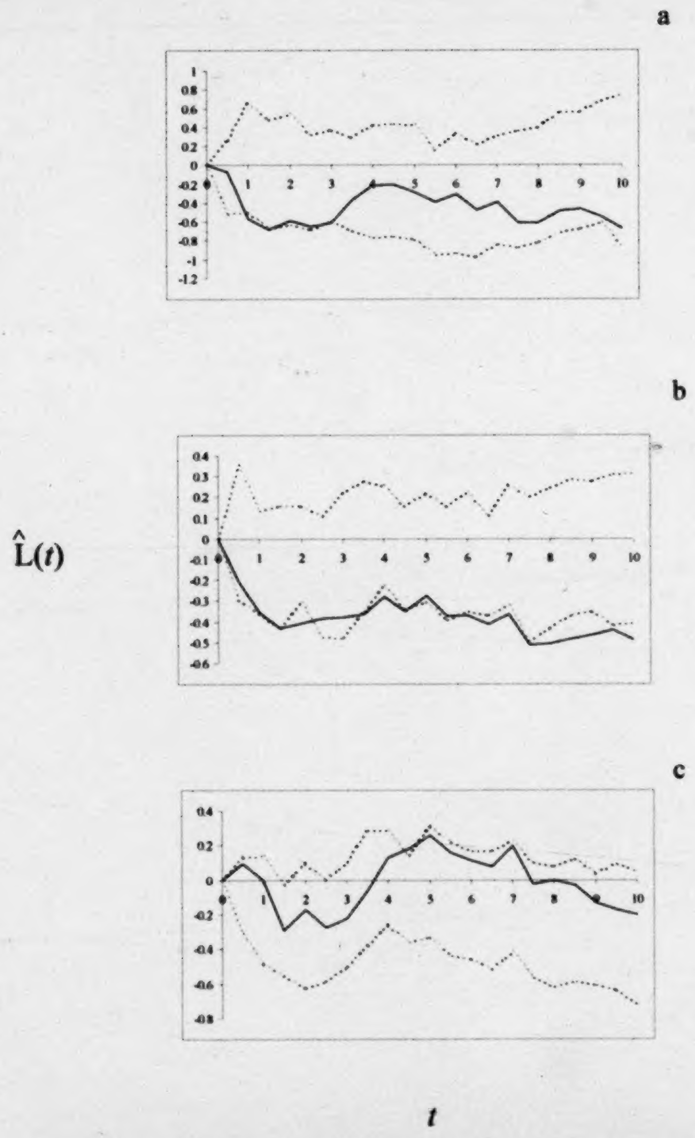

Figure 3.19: Results of Ripley's K bivariate graphs for plot 1. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of t and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

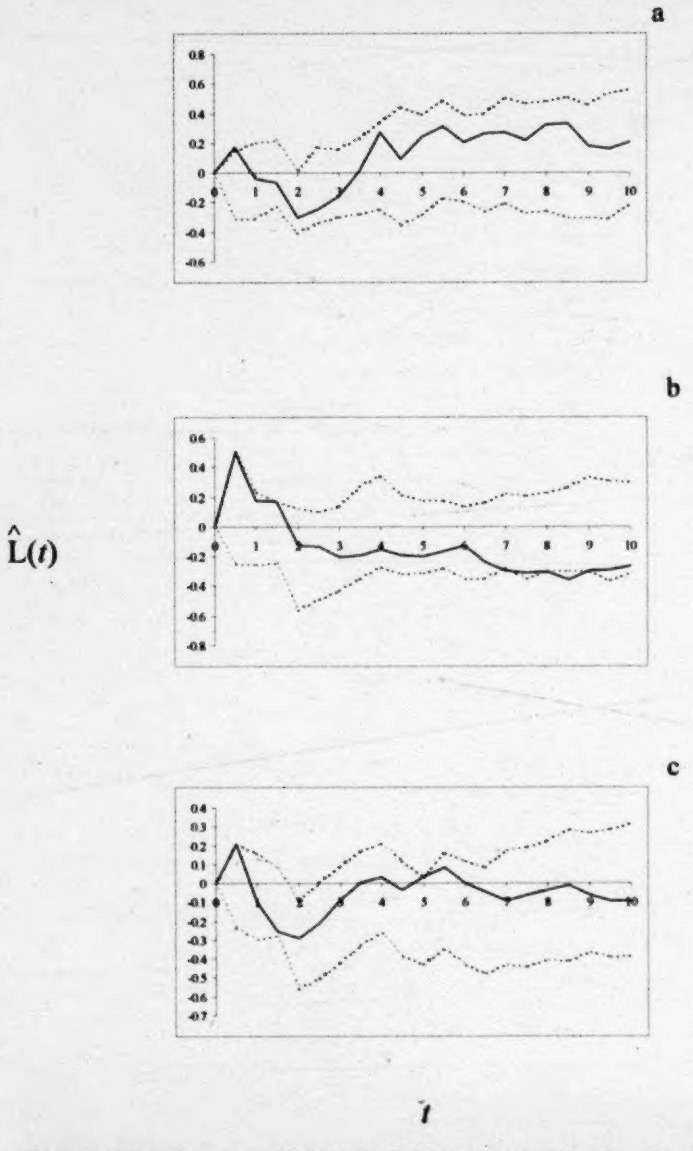

Figure 3.20: Results of Ripley's K bivariate graphs for plot 2. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

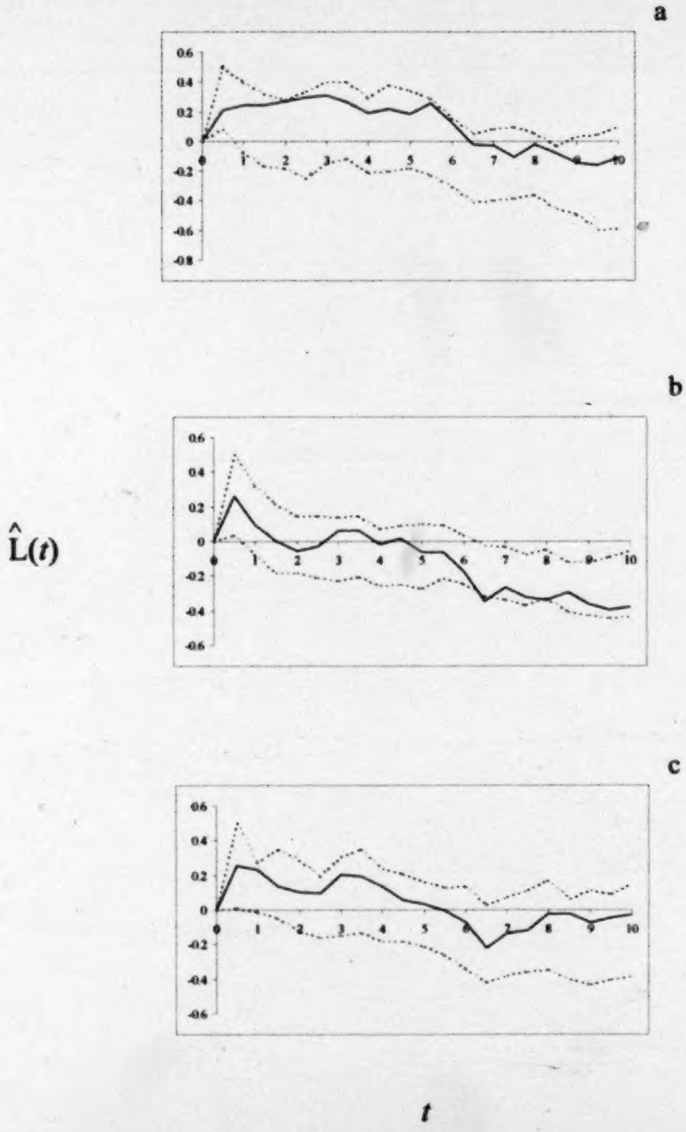

Figure 3.21: Results of Ripley's K bivariate graphs for plot 3. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

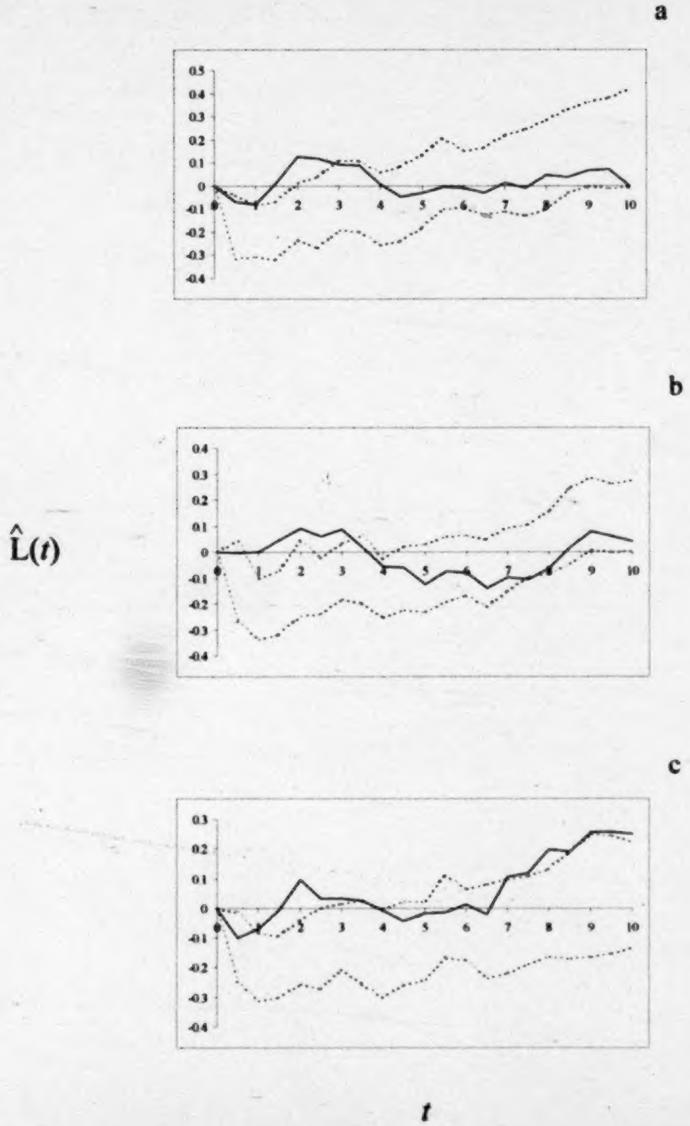

Figure 3.22: Results of Ripley's K bivariate graphs for plot 4. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

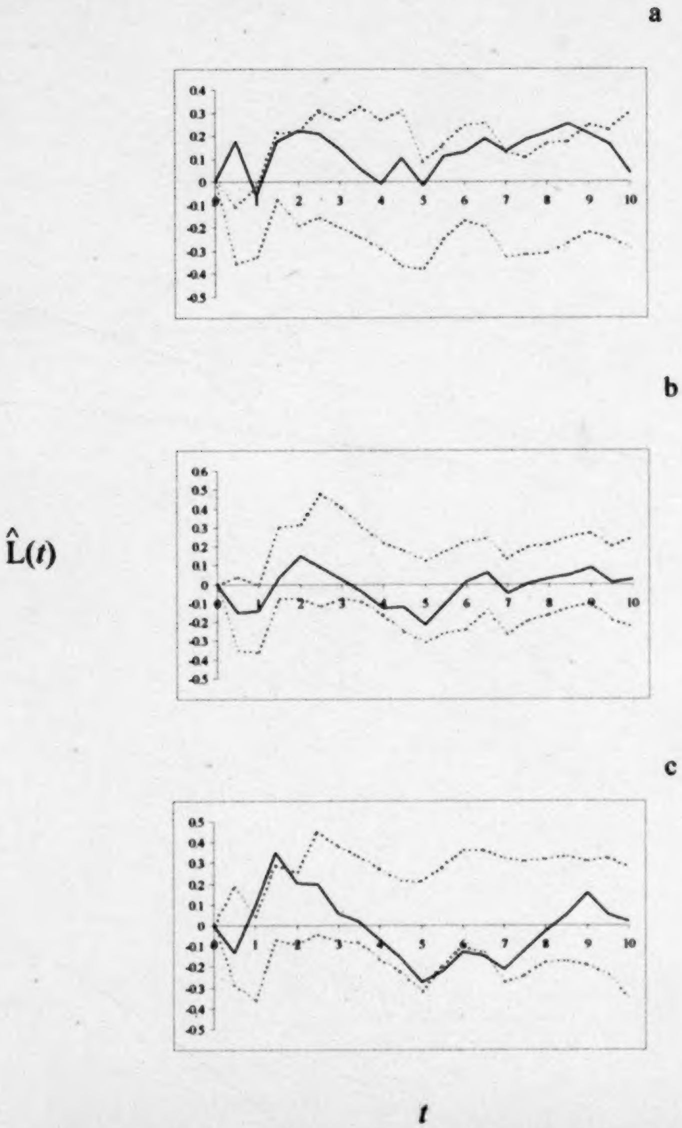

Figure 3.23: Results of Ripley's K bivariate graphs for plot 5. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of t and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

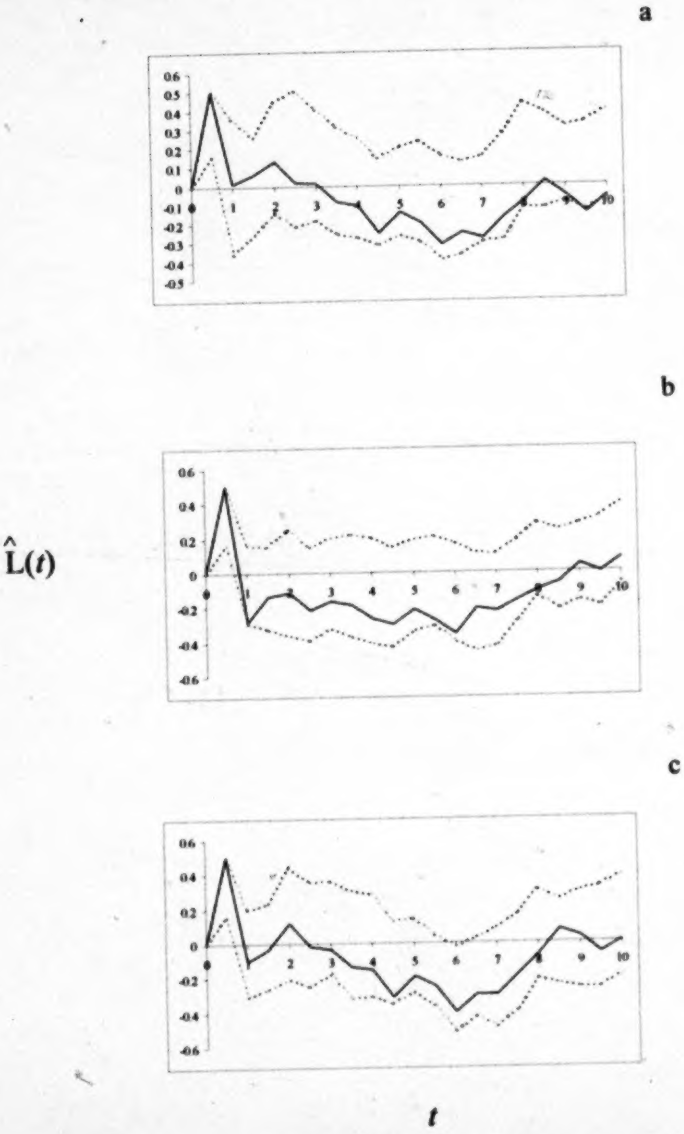

Figure 3.24: Results of Ripley's K bivariate graphs for plot 6. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

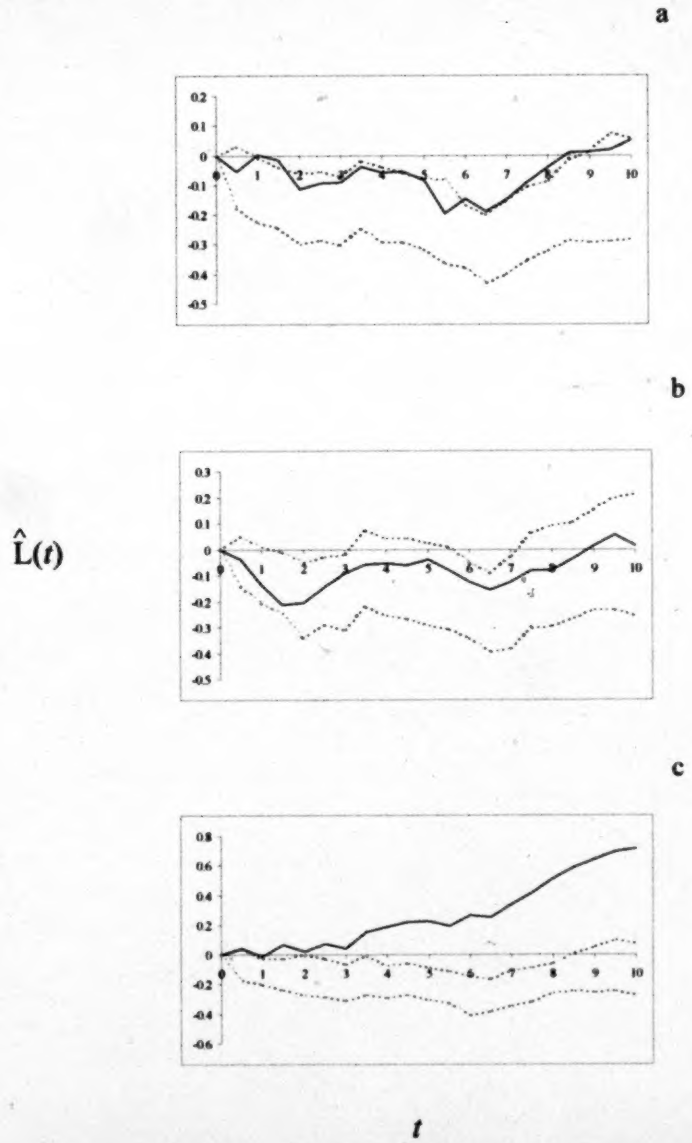

Figure 3.25: Results of Ripley's K bivariate graphs for plot 7. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

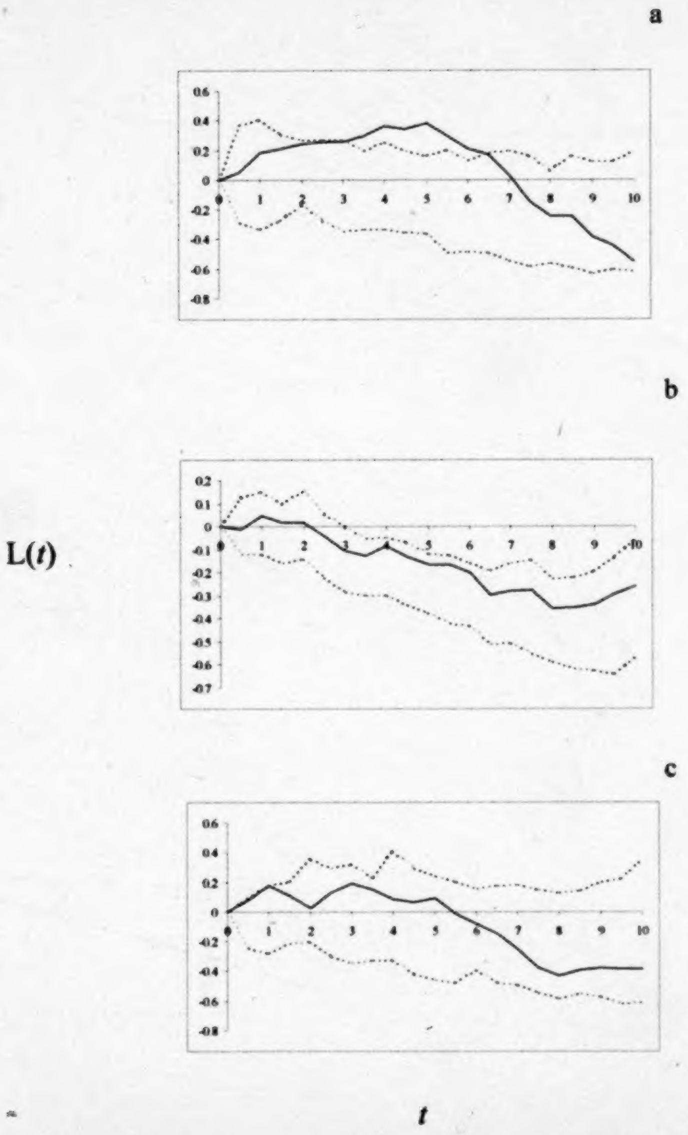

Figure 3.26: Results of Ripley's K bivariate graphs for plot 8. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

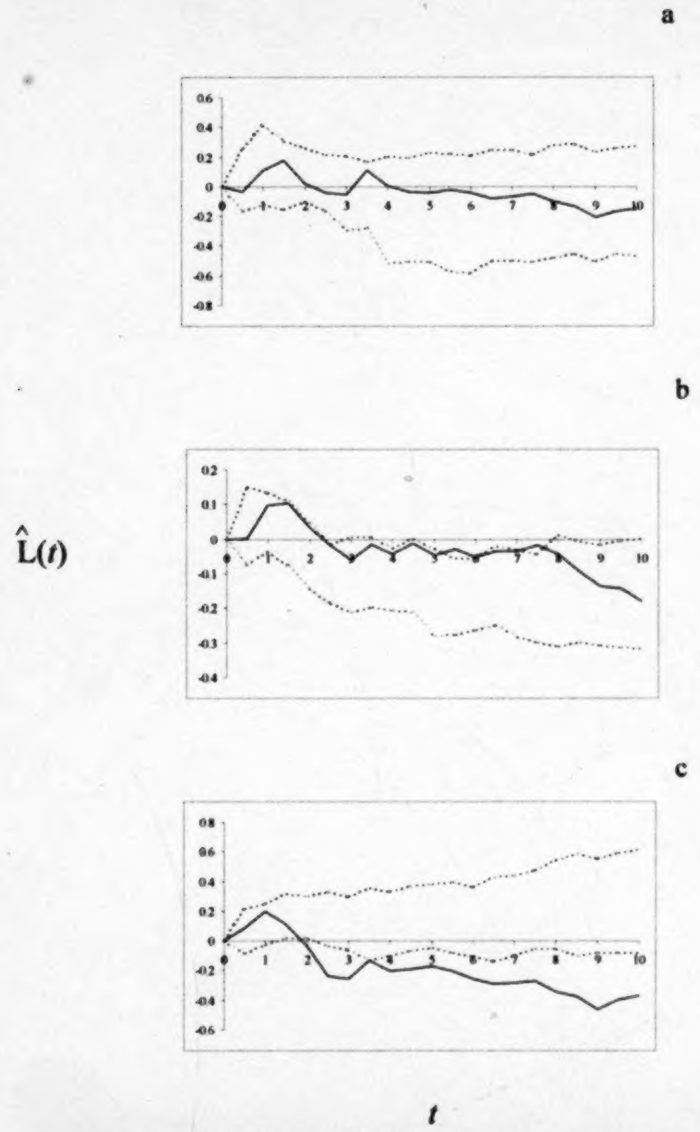

Figure 3.27: Results of Ripley's K bivariate graphs for plot 9. Alpha = 0.05. The solid single line represents the estimate of the point pattern at scales of *t* and the dotted lines are the confidence intervals. If the solid line falls below the lower confidence interval it indicates a pattern of aggregation alternatively if the solid line is above the upper confidence interval it indicates a pattern of segregation. (a) bivariate pattern of broadleaf vs coniferous tees, snags, and stumps (b) bivariate pattern of healthy trees vs unhealthy trees, snags and stumps (c) bivariate pattern infected vs uninfected trees, snags, and stumps.

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Chapter Four

General Discussion and Conclusions

Host preference of Armillaria sinapina

The results from this study support previously made statements that *Armillaria sinapina* prefers broadleaf hosts (e.g. Mallett 1990). In all plots sampled in this study, *A. sinapina* was more likely to be found infecting the roots and root collars of broadleaf tree species (Chapter Two). There are no studies that have directly addressed the possible reasons for this preference.

The coniferous tree species sampled in this study appeared to be resistant to infection by *A. sinapina*, relative to broadleaf trees (Chapter Two). Kile *et al.* (1991) suggested that as conifer trees age, they become more resistant to infection and disease caused by *Armillaria* species. Mallett and Hiratsuka (1988) showed that *A. sinapina* was a virulent pathogen of young lodgepole pine in a greenhouse experiment. However, data showing exactly how coniferous trees are more resistant to *A. sinapina* infection than broadleaf trees and how resistance changes with age have not been published.

This results of this study also suggested that *Armillaria sinapina*, as opposed to *A. ostoyae*, is the dominant, and perhaps the only, species of *Armillaria* associated with woody substrates in the northern mixedwood boreal forest types that were sampled.

Armillaria sinapina*: the pathogen*

The results of this study suggest that *Armillaria sinapina* is a ubiquitous, weak, long-lived pathogen in mixedwood forests. In the two plots with the greatest percentages of broadleaf trees, snags, and stumps, greater than 90% of healthy asymptomatic trees were infected at the root collar by *A. sinapina*. Even though these trees were infected, *A. sinapina* was not causing 'disease', where 'disease' is defined as the visual expression of symptoms by the host due to the presence of the pathogen (British Federation of Plant Pathologists 1973). It may be possible that seemingly healthy trees with *A. sinapina* infection may be suffering growth loss. Mallett and Volney (1999) examined growth of lodgepole pine trees with and without *Armillaria ostoyae* infections and found that infected trees suffered growth losses in height and volume. However, there are no empirical data available concerning possible growth loss to broadleaf trees infected with *Armillaria sinapina*. In the pure broadleaf plots in this study it would be very difficult to find a broadleaf tree that was not infected with *A. sinapina*, therefore making comparisons between infected and uninfected trees near impossible.

In addition, at small scales (less than 10 m) the spatial pattern of infection by *A. sinapina* was mostly clumped but in the broadleaf dominated plots, this clumping was likely to have been caused by clumping of the broadleaf trees. These findings are supported by previous statements that *A. sinapina* is a weak or 'mild' pathogen and usually saprophytic (Morrison *et al*. 1985, Bérubé and Dessureault 1988, Dumas 1988, Shaw and Loopstra 1988, Banik *et al*. 1995). It has been suggested that *Armillaria* species in general are potentially long-lived and exist as a stable mosaic in the forest landscape, interacting and having an influence on the age and genetic structure, and the composition of plant populations over long time periods reaching centuries (Alexander, 1992, Bruhn *et al.* 2000). It has been thought for a long time that root rot exists in forested areas with relatively little effect, prior to additional disturbances such as insects, other fungal diseases, harvesting and fire (Stanosz and Patton 1991).

In forests of North America, *Armillaria sinapina* is thought to be less pathogenic than *A. ostoyae* especially in regards to conifer infection and disease (Morrison *et al* 1985, Dumas 1988, Kile *et al.* 1991). *Armillaria sinapina* was found to be widely distributed in south-eastern Alaska forests but was found not to be a major factor in killing regenerating stands (Shaw 1981, Shaw and Loopstra 1988). In a laboratory experiment, white spruce and lodgepole pine seedlings were infected by *A. sinapina* but *A. sinapina* isolates did not cause mortality (Mugala *et al.* 1989). In contrast, a laboratory study showed that lodgepole pine seedlings inoculated with *A. ostoyae* and *A. sinapina* were more susceptible to infection and death by *A. sinapina* (Mallett and Hiratsuka 1988). In addition, Mallett (pers. comm., unpublished data) has repeated these experiments several times and has found *A. sinapina* causing mortality in white spruce.

There is much research showing that the behaviour of pathogens may change with the local abiotic and biotic conditions (e.g. Jarosz and Davelos 1995, Deacon 1996). Wiensczyk *et al.* (1997) showed that infection levels of *A. ostoyae* in black spruce (*Picea mariana* (Mill.)) plantations depended on tree age, soil clay content, and soil texture. Similar findings have been shown for *Armillaria ostoyae* in Alberta (e.g. Mallett and Maynard 1998). Therefore, there is a good chance that the seemingly innocuous presence of *A. sinapina* in the plots in this study may not continue in the long term. If environmental conditions for the fungus changed somehow, such as if soil moisture increased or if many of the trees became weakened or damaged, perhaps *A. sinapina* would become a more aggressive pathogen. It would be in a good position to do so, considering the large proportion of trees that were infected and the large amounts of rhizomorphs present in the forest floor. This is supported by Morrison (1989) who stated that the ecological strategy of a weakly pathogenic or saprophytic species of *Armillaria* may be to produce many rhizomorphs that grow epiphytically on the roots and root collars of trees. These rhizomorphs would then be in a good position to infect and cause disease when the trees are weakened by other factors such as insect attack (Morrison 1989).

Armillaria sinapina*: the saprophyte*

The first fungi to appear in the succession of decaying wood are usually soil fungi, which colonize non-lignified cell walls (Lumley 1999). Basidiomycetes, which include *Armillaria* species, are thought to colonize next, degrading lignin and cellulose in the wood cell walls. It is believed that secondary molds and fungi that show a variety of enzymatic capacities, which are unable to break down lignin and cellulose but that benefit from the residuals of the destroyed lignin and cellulose, occur last (Lumley 1999).

The role that *A. sinapina* specifically plays in the decomposition of downed woody material (DWM) and its occurrence in the order of succession of fungal species has not been documented. In this study, *A. sinapina* was found to be colonising broadleaf logs of a wide range of stages of decay from undecayed to the last stages of decay, where logs were very soft, without branches, little bark, and were almost indistinguishable from soil. In addition, many trap-logs, recently killed aspen saplings, were colonized by *A. sinapina* especially in broadleaf dominated stands. Fans of mycelia were found growing beneath the bark of these recently killed logs, obviously utilizing the sugars available in the cambium layer. This suggests that *A. sinapina* may contribute to early stages of decay, which would fit the pattern of log decomposition outlined above.

The results of this study also suggest that the presence of *Armillaria sinapina* on DWM is, at least in part, determined by whether or not it was present on the tree before it fell. This is important because it emphasises the role of vegetative spread over basidiospores and suggests that it is a long-lived species (Redfern and Filip 1991).

Presence and abundance of Armillaria sinapina *rhizomorphs in the forest floor*

The results of this study suggest that in this system, at the scale of this study (within 40 × 40 m plots), *A. sinapina* uses the forest floor as its primary medium for dispersal, utilising rhizomorphs rather than basidiospores (air dispersal). First, trap-logs and forest floor samples showed that rhizomorphs of *A. sinapina* were present in the forest floor in all of the nine plots sampled. Second, of the environmental variables measured, very few predicted either the presence or abundance of *Armillaria sinapina* in forest floor samples. This may suggest that rhizomorphs are relatively tolerant of different conditions and are in effect 'searching' for available substrates. Redfern and Filip (1991) state that rhizomorphs of *Armillaria* spp can tolerate a wide range of soil conditions. Third, in all instances in this study where infection was recorded in trees, a rhizomorph was traced from the forest floor, through the bark and into the cambium where the site of infection occurred.

However, this study did not address intra-specific genetic variation within the sampled *A. sinapina* mycelia. Until sampling is done at this level, it is impossible to say with certainty that sexual reproduction by *A. sinapina* does not play an important role in its dispersal throughout this system.

Tree health assessments

From this study, it is difficult to conclude that *A. sinapina* is playing a major role in causing broadleaf death because healthy and dead broadleaves were equally infected. Although it is clear that *A. sinapina* definitely prefers to colonize broadleaf hosts further study is needed to assess whether an infection by *A. sinapina* in a healthy broadleaf tree ultimately leads to the death of the tree. It appears that *A. sinapina* is a common saprophyte of broadleaf trees, snags, stumps and downed woody material and at times could change roles to be a pathogen of broadleaf trees. This ability to switch roles from a saprophyte to a pathogen could be tested in a laboratory situation.

For conifers, however, my results suggest that *A. sinapina* may be playing a role in the tree decline or death. When healthy and dead coniferous trees were compared dead coniferous trees were more likely to be infected by *A. sinapina*. Further, recall that the proportion of conifer DWM pieces that were colonized at the base was higher than the proportional colonization of trees, snags, and stumps. In this study, *A. sinapina* appears to be playing a role in conifer tree death.

Future research directions

One way this project could be extended is to look at exactly what influences whether or not *Armillaria sinapina* causes infection and/or disease in any given tree. This requires detailed study of the physiological interaction between the fungus, the tree, and their environment (both biotic and abiotic).

The temporal dynamics of this species should also be considered. If this 'disease' plays a long-term role in these forest systems, then expanding this sort of study over long time scales would be necessary to understand its ecology. Unfortunately, destructive sampling is currently required to record the presence of infection. However, there may be other, non-destructive options that could be further developed, such as trap-log methods (Mallett and Hiratsuka 1985), or sniffing dogs (Swedjemark 1989). A way to get around this would be to investigate similar forest types at different ages.

Different scales should be considered. Unfortunately this study was limited by time constraints and the plot sizes were established at a size that was practical for sampling. However, the results suggest that if larger scales (i.e. larger plots) were considered, the spatial pattern of the fungus may be better described at a landscape scale.

How *Armillaria sinapina* responds to disturbances such as harvesting versus fire should be considered. This would provide valuable information for people interested in the regeneration of trees following disturbance events.

Forest management issues and applications

There have been suggestions for management strategies of *Armillaria* root disease following harvesting. Fumigation has been shown to effectively kill *Armillaria* root disease in stumps and root systems but this method is costly and it is unknown how this technique influences the surrounding non-targeted biota (Filip and Roth 1977). In addition, fumigants are now banned and therefore would not be viable option at this time (Ken Mallett, pers. comm.). Others have suggested biological control of *Armillaria* root disease by inoculating stumps with antagonistic wood decay fungi (Pearce and Malajczuk 1990). Other fungi will grow rapidly and colonize a portion of the stump and root system that is not occupied by *Armillaria* and therefore limits the size of the available food base for *Armillaria*. However, the results of this study show that healthy trees were often infected and so perhaps *A. sinapina* is helping healthy trees by limiting the ability of other fungi to infect the tree. This may seem as a bit of a leap but Shaw and Loopstra (1988) suggested that the presence of *A. sinapina* and NABS IX in stump and root wood might act as a biocontrol of another root rot pathogen, *Heterobasidion annosum*, by deterring its spread from stumps to adjacent trees.

The current recommendation for silvicultural management of *A. ostoyae* in British Columbia is to reduce its inoculum potential by removing stumps following conventional harvesting (Morrison and Mallett 1996). In sites where removing stumps is not feasible, planting less-susceptible species to *Armillaria* species infection is considered the best option (Morrison and Mallett 1996). It is then imperative to know which species of

Armillaria are present on site pre-harvest, what tree hosts are susceptible to these *Armillaria* species and where you would predict the presence of these *Armillaria* species.

Results from this study have important implications for boreal forest management in North-western Alberta. *A. sinapina* was the only species of *Armillaria* identified in all nine plots. These results show that *A. sinapina* is commonly found infecting broadleaf trees, snags, and stumps in broadleaf stands. If *Armillaria sinapina* is an important pathogen, then this study shows that it may be important to consider the status of asymptomatic trees in a pre-harvest disease survey, as this may vary differently than the numbers of dead and dying trees.

In order to manage these forests it is necessary to know which *Armillaria* pathogens are present and what environmental factors predict their occurrence. Once this baseline biological and ecological information is established further ecological research can proceed. With enhanced knowledge of the role of *Armillaria* in forested ecosystems, more feasible management plans can be established.

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Personal communication

Dr. Ken Mallett, 5320 122st, Northern Forestry Centre, Edmonton, T6H 3S5, Alberta, Canada

APPENDIX I: Table summarising data for the nine 40 x 40 m plots. Data are: the total number of trees per plot (N_{total}), The total number broken down into healthy trees, declining trees, and dead trees (including snags and stumps) (N_{hl}), deciduous or coniferous (N_{sp}), and by infected, or not infected (absent) (N_{in}). For each of these groups the average height, basal diameter, and diameter at breast height (DBH = 1.3m) ± standard deviations are given.

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
1	153	Healthy	119	Deciduous	8	Infected	6	23.9±1.6	37.4±6.5	30.8±5.8
						Absent	2	24.2±4.2	37.1±17.8	30.4±14.2
				Coniferous	111	Infected	5	27.1±3.6	41.5±11.5	32.6±7.8
						Absent	106	22.4±8.4	32.1±15.2	26.6±12.4
		Declining	8	Deciduous	0	Infected	0	-	-	-
						Absent	0	-	-	-
				Coniferous	8	Infected	0	-	-	-
						Absent	8	14.7±11.1	14.8±14.8	14.2±13.1
		Dead	26	Deciduous	3	Infected	3	3.4±4.8	20.1±0.8	-
						Absent	0	-	-	-
				Coniferous	23	Infected	10	8.4±4.9	15.8±9.7	11.2±7.7
						Absent	13	6.4±3.7	11.3±6.5	6.1±22.9

Appendix I continued

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
2	179	Healthy	130	Deciduous	29	Infected	16	25.15±6.77	43.3±9.4	33.6±7.1
						Absent	13	6.15±9.88	6.09±12.35	4.5±10.5
				Coniferous	101	Infected	4	23.7±1.81	30.0±1.97	22.9±1.38
						Absent	97	17.9±12.1	25.8±18.2	18.6±13.5
		Declining	4	Deciduous	1	Infected	1	17.3	29.28	24.83
						Absent	0	-	-	-
				Coniferous	3	Infected	0	-	-	-
						Absent	3	12.76±.98	16.55±1.93	12.31±0.97
		Dead	45	Deciduous	4	Infected	3	3.25±3.24	40.42±26.11	35.65±21.16
						Absent	1	0.22	22.28	-
				Coniferous	41	Infected	20	8.29±7.61	28.95±12.01	19.31±7.11
						Absent	21	2.4±4.9	28.9±10.67	20.1±3.1

Appendix I continued

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
3	198	Healthy	132	Deciduous	8	Infected	6	27.8±2.8	41.9±7.3	29.9±4.5
						Absent	2	23.4±7	36.8±3.4	28.2±0.7
				Coniferous	124	Infected	8	22.9±5.5	33.6±13.1	26.9±11.8
						Absent	116	17.2±8.1	25.4±10.8	20.2±8.8
		Declining	2	Deciduous	1	Infected	0	-	-	-
						Absent	1	20.6	23.2	18.8
				Coniferous	1	Infected	0	-	-	-
						Absent	1	4.5	6.4	4.5
		Dead	64	Deciduous	31	Infected	30	4.1±7.7	26.1±9.3	25.1±6.7
						Absent	1	20.95	43.93	32.15
				Coniferous	33	Infected	20	9.5±8.1	28.1±9.8	21.8±7.8
						Absent	13	5.7±6.7	22.4±10.1	13.7±9.2

Appendix I continued

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
4	275	Healthy	165	Deciduous	29	Infected	25	25.6±5.7	33.8±11.8	28.5±8.5
						Absent	4	24.4±1.4	19.1±22.1	28.3±0.5
				Coniferous	136	Infected	8	23.1±7.5	31.9±13.3	26.7±11.9
						Absent	128	2.01±0.5	3.6±0.9	1.5±0.9
		Declining	34	Deciduous	9	Infected	9	24.2±4.5	30.6±5.1	25.6±5.0
						Absent	0	-	-	-
				Coniferous	25	Infected	2	18.9±0.8	20.5±1.1	16.4±0.7
						Absent	23	5.5±6.2	13.5±7.9	9.3±7.4
		Dead	76	Deciduous	43	Infected	36	3.7±6.9	23.3±6.3	22.6±6.1
						Absent	7	0.9±0.9	21.5±4.5	
				Coniferous	33	Infected	19	11.5±5.4	17.7±9.6	15.4±8.2
						Absent	14	7.1±4.7	16.9±9.9	10.6±6.5

Appendix I continued

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
5	143	Healthy	89	Deciduous	27	Infected	26	23.9±2.4	35.8±8.7	28.6±7.9
						Absent	1	25.6	28.7	22.3
				Coniferous	62	Infected	4	23.6±7.2	39.9±14.9	31.9±11.9
						Absent	58	22.8±7.8	35.3±14.4	28.2±12.4
		Declining	5	Deciduous	1	Infected	1	22.4	30.6	23.6
						Absent	0	-	-	-
				Coniferous	4	Infected	1	4.3	6.4	4.1
						Absent	3	12.3±6.8	21.4±5.3	18.5±7.0
		Dead	49	Deciduous	25	Infected	22	3.7±6.1	27.9±10.7	23.5±10.1
						Absent	3	0.14	10.6±4.04	-
				Coniferous	24	Infected	19	8.9±7.7	23.5±11.0	15.7±7.3
						Absent	5	4.4±6.5	26.3±10.3	-

Appendix I continued

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
6	134	Healthy	79	Deciduous	8	Infected	8	26.3±2.1	49.2±6.2	35.5±3.9
						Absent	0	-	-	-
				Coniferous	71	Infected	0	-	-	-
						Absent	71	14.8±11.1	24.9±23.1	19.4±18.5
		Declining	7	Deciduous	3	Infected	3	24.2±1.7	43.1±2.2	30.6±2.2
						Absent	0	-	-	-
				Coniferous	4	Infected	0	-	-	-
						Absent	4	9.1±5.6	14.2±7.8	10.1±7.9
		Dead	48	Deciduous	39	Infected	37	4.1±5.9	37.5±12.6	30.5±8.9
						Absent	2	0.38	34.2	-
				Coniferous	9	Infected	2	8±0.6	13.8±7.4	10.8±5.4
						Absent	7	9.6±9.5	23.2±28.7	16.5±20.2

Appendix I continued

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
7	278	Healthy	181	Deciduous	79	Infected	61	22.5±7.9	31.8±9.1	27.1±7.7
				Coniferous	102	Absent	18	22.7±4.7	30.5±7.3	24.6±5.5
						Infected	9	16.8±2.4	23.3±5.8	18.5±4.6
		Declining	13	Deciduous	11	Absent	96	13.3±6.2	16.3±7.5	13.3±6
						Infected	8	23.7±2.1	26.1±3.5	21.2±2.6
				Coniferous	2	Absent	3	23.95±3.2	28±12.1	21.5±7.4
						Infected	0	-	-	-
		Dead	84	Deciduous	82	Absent	2	5.8±0.8	5.9±0.7	4.9±1.1
						Infected	67	7.1±8.9	20.1±7.7	16.9±6.4
				Coniferous	2	Absent	15	5.9±8.7	18.1±8.1	15.1±7.2
						Infected	0	-	-	-
						Absent	2	1.97	8.3	-

Appendix I continued

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
8	237	Healthy	139	Deciduous	115	Infected	112	22.2±3.3	29.6±7.6	24.9±5.2
						Absent	3	15.7±12.1	19.1±16.0	15.5±13.7
				Coniferous	24	Infected	1	3.7	6.1	4.8
						Absent	23	3.2±1.2	6.9±7.5	4.2±4.3
		Declining	8	Deciduous	8	Infected	8	14.8±6.5	18.7±4.3	17.2±3.2
						Absent	0	-	-	-
				Coniferous	0	Infected	0	-	-	-
						Absent	0	-	-	-
		Dead	90	Deciduous	90	Infected	89	3.3±6.01	18.1±5.4	-
						Absent	1	1.07	19.7	-
				Coniferous	0	Infected	0	-	-	-
						Absent	0	-	-	-

Appendix I continued

Plot	N_{total}	Health	N_{hl}	Species	N_{sp}		N_{in}	Average Height (m) ± std. dev.	Average Basal diameter (cm) ± std. dev.	Average DBH (cm) ± std. dev.
9	314	Healthy	153	Deciduous	128	Infected	119	21.8±5.5	24.4±6.23	21.1±5.43
						Absent	9	13.1±9.02	13.9±9.72	11.8±9.13
				Coniferous	25	Infected	0	-	-	-
						Absent	25	14.6±1.8	19.8±1.1	16.4±1.6
		Declining	35	Deciduous	35	Infected	34	20.5±6.9	21.07±4.54	-
						Absent	1	15.7	14.3	11.8
				Coniferous	0	Infected	0	-	-	-
						Absent	0	-	-	-
		Dead	126	Deciduous	126	Infected	117	4.3±7.2	16.1±5.1	-
						Absent	9	1.4±3.1	14.37±5.7	-
				Coniferous	0	Infected	0	-	-	-
						Absent	0	-	-	-

Appendix II: A detailed description of both the univariate and bivariate Ripley's K function used in Chapter 3 to analyse the point patterns of infected trees.

Theoretical background on the Ripley's K function

For a one-dimensional set of numbers, the mean (μ) and variance (σ^2) are the first and second moments (variance can be defined as a measure of dispersion about some value in this case the mean) (Sterner *et al.* 1986). For a two-dimensional set of numbers density (λ) and covariance (κ) are the first and second moments (covariance can be defined as a measure of the distribution of the distances between points) (Sterner *et al.* 1986). Ripley (1976, 1977) developed density functions or a second order statistic for two dimensional sets of numbers which used the structure of the covariance's which measures the distribution of the distances between all points-to-points. This second order statistic can be reduced to a function called K(t), where t is a distance between 0 and ∞. If λ is defined as the density of points per unit area and a circle is centered randomly on a chosen point, the expected number of other points within radius t is λ multiplied by the function of K(t) (Dale 1999). Following Ripley (1976, 1981) K(t) is estimated by K(t):

$$\hat{K}(t) = A \sum_{i \neq j}^{n} \sum^{n} w_{ij} I_t(u_{ij}) / n^2 \qquad 1$$

where A is the area of the plot, w_{ij} is an edge correction term which follows Haase 1995 where it assumes that the area outside the plot boundary has a point density and distribution pattern similar to that closest to the edge within the plot. $I_t\,(u_{ij})$ is the counting function, and I_t takes the value of 1 when the distance between *i* and *j* is less than or equal to radius *t*, otherwise it is counted as 0. The distance between the two points *i* and *j* can be calculated by $u_{ij} = \sqrt{(x_i - x_j)^2 + (y_i - y_j)^2}$ and then compared to a value of *t* (Haase 1995). Finally, *n* is the number of events in the area A. The intervals of *t* or circle size is user defined and the estimate of K(t) is recalculated for each value of *t* chosen. For each circle size *t*, a sum of the pairs of points is calculated and is divided by the total number of points squared (fig 1)

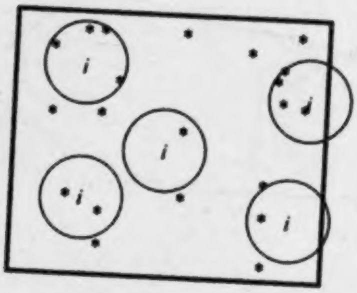

Figure 1: This figure illustrates the counting function for a value of *t* of the univariate estimate of Ripley's K function. Circles are centered on points, *i*. Pairs of points *i* and * are counted for each circle. The upper left-hand circle would have a value of 4 and the circle in the centre would have a value of 1 and so on. Circles that fall outside the plot boundary are subjected to the edge correction noted above.

Ripley's K(*t*) analysis is used to test for the overall type of pattern as it departs from a Poisson process (Moeur 1993). The null hypothesis for a Poisson model is that the points are distributed randomly and independently in space which implies no interaction between points. $K(t) = \pi t^2$ if points are randomly arranged in a Poisson distribution. A much easier way of graphing and interpreting the estimates of the Ripley's K function is to perform a square root transformation which linearizes $\hat{K}(t)$, stabilizes the variance and has an expected value of 0 under a Poisson process which gives:

$$\hat{L}(t) = t - \sqrt{\hat{K}(t)/\pi}$$

A plot of $\hat{L}(t)$ versus t is used to investigate the type of point patterns at different scales of t. Significantly large positive values (over the line) indicate the points are over-dispersed and a significantly large negative value (under the line) indicates the points are clumped.

Bivariate Ripley's K function (second-order analysis)

The bivariate version of Ripley's K function is often recommended as a method for investigating the bivariate spatial pattern of mapped points (Upton and Fingleton 1985, Andersen 1992, Dale 1999). This method is based on distances between all pairs of points. Different sizes of circles are centered on each type of point and the numbers of points of the other species are counted. The counting function $I_t(i, j)$ takes the value of 1 when $d_{ij} \leq t$, i.e. the distance between the center point i (species 1) and the counted point j (species 2) is less than or the same as the radius t.

The estimates of the bivariate Ripley's K function are:

$$\hat{K}_{1,2}(t) = A \sum_{i}^{n_1} \sum_{j}^{n_2} w_{ij} I_t(i,j)/n_1 n_2 \text{ and}$$

$$\hat{K}_{2,1}(t) = A \sum_{i}^{n_1} \sum_{j}^{n_2} w_{ji} I_t(i,j)/n_1 n_2 .$$

Where A is the area of the plot, w_{ij} is the edge correction, $I_t(i,j)$ is the counting function and $n_1 n_2$ is the product of the numbers of species 1 and 2. $\hat{K}_{12}(t)$ and $\hat{K}_{21}(t)$ are combined to give:

$$n_2 \hat{K}_{1,2}(t) + n_1 \hat{K}_{2,1}(t)/(n_1 + n_2)$$

To examine the bivariate spatial pattern

$$\hat{L}(t) = t - \sqrt{}[n_2 K_{1,2}(t) + n_1 K_{2,1}(t)]/\pi(n_1 + n_2)$$

Is plotted as a function of t. Significantly large positive values (over the line) indicate the two species are segregated and a significantly large negative value (under the line) indicates the two species are aggregated.

Ripley's K detects the aggregation and segregation of points. It can be used to examine the size and spacing of clumping, but it does not detect true scales of aggregation and segregation. One inconvenience in using the method is that circles centered on points close to the edge are likely to contain a lower number of points than expected. To correct for this, an edge correction must be applied, of which there are several alternatives (Ripley 1981, Diggle 1983, Getis and Franklin 1988, Haase 1995). For example, Getis and Franklin (1988) use an edge correction that is based on the assumption that any area within radius t, but outside the study area, has a similar number of points as the circle area inside the study area. A more time consuming alternative to using an edge correction is to map a buffer area around the actual study area so that in the analysis none of the circles fall outside the edge (Dale 1999).

Another drawback to this method is that the points within the circles are averaged over all circles of a particular size. This means that particular circles of a given radius t may have more low and high counts than expected but $\hat{L}(t)$ is still close to 0. This method does not identify areas within the study plot with a high density or low density of points. Getis and Franklin (1988) use contour lines for univariate point patterns to indicate areas of high or low point density. This method could possibly be extended in a bivariate case.

Because all the circles are centered on points, areas without points will not be detected. To avoid this effect, Getis and Franklin (1988) randomly placed some circles in empty areas. Again, this may possibly be extended to the bivariate case. The range of data that can be effectively interpreted using Ripley's K is limited as it assumes that the pattern has stationarity and is isotropic. However, there is a way to examine the pattern for anisotropy. Each circle is split into sections and the K function is recalculated for sections in different directions (Dale 1999).

Null hypotheses and Randomisation tests

Instead of using a test statistic to calculate departures from a random distribution, Monte Carlo simulations were performed. The observed distributions of $\hat{L}(t)$ are compared with values obtained from the multiple randomisations generated from either a model of complete spatial randomness for univariate data or a model of independent distributions for bivariate data. Both these types of randomisation tests were used to generate 95% confidence intervals from 100 simulations.

The null hypothesis of complete spatial randomness was tested using the univariate Ripley's K function. The significance of this test was determined using randomisation tests, where the positions of all the points were randomised, but the number of points was kept constant and the function recalculated many times.

The null hypothesis of independent distributions of the two species was tested, using the bivariate Ripley's K function. The significance of this test was determined using randomisation tests, where the labels of the points (species 1 and species 2) were randomised, but the positions of the points were kept constant and the function recalculated many times.

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